(54) Title: OPTICAL BIOSENSOR SYSTEM

(57) Abstract

An optical biosensor system using internal reflection versus angle of incidence determination for the detection of biomolecules, said system comprising a sensor unit (10) with at least two sensing surfaces (39A-D), a source of light (1), and lens means (2) for forming a convergent beam of light which is focused in wedge-shape fashion to form a streak of light (5) extending transversely over all the sensing surfaces; a photodetector device (7) in the form of a two-dimensional matrix of individual photodetector; optical imaging instrumentation in the form of an anamorphic lens system (6) for the purpose of imaging rays of reflected light from the sensing surfaces on each its own column of photodetectors, so that for each sensing surface there is a corresponding set of columns of photodetectors; and an evaluation unit (8) for determining the minimum reflectance or the resonance angle at each of the sensing surfaces. The invention also relates to a method for calibrating the biosensor system, a method for correcting for baseline drift as well as a method for temperature regulation of thermostat means in the biosensor system.

Data communication

OPTICAL BIOSENSOR SYSTEM

The present invention relates to an optical multi-analyte biosensor system employing the principle of internal reflection of polarized light for use in biological, biochemical and chemical analysis and in particular for detecting a specific molecule, for example antigens. The detection method used in the biosensor system may be based on the evanescent wave phenomenon at total internal reflection, such as surface plasmon resonance (SPR), critical angle refractometry, total internal reflection fluorescence (TIRF), total internal reflection phosphorescence, total internal reflection light scattering, and evanescent wave ellipsometry. Furthermore, the detection method may be based on Brewster angle reflectometry. This optical biosensor system is one of generally the type as set forth in the preamble of the attached claim 1.

The main advantage of the internal reflection based techniques is that the sensitivity region for the specific substance is restricted to the extension length of an evanescent wave, i.e. the depth of an electromagnetic wave penetrating into the liquid medium from the sensing surface side. Consequently, there will be a minimum of influence on the response connected to specifically bound analyte molecules from non-bound sample molecules. Moreover, the penetration depth of the evanescent wave for a totally reflected ray of light depends on the angle of incidence for the ray. For a comprehensive treatment of the concept of internal reflection it is referred to Mirabella and Harrick, Internal Reflection Spectroscopy, Harrick Scientific Corporation, N.Y. 1985.

The optical response induced by either the primary evanescent wave, or by a secondary evanescent wave excited in turn by said primary evanescent wave, may be measured as changes in

the reflectance or in the state of polarization of the incident light wave upon reflection, or as the fluorescence or phosphorescence or light scatter of radiation, as a result of a specific substance interaction with a sensing layer at the sensing surface.

The optical response related to the specific substance may be measured as the reflected intensity as a function of angle of incidence of p-polarized light, being applicable for surface plasmon resonance, Brewster angle reflectometry, and critical-angle refractometry, in that the angle of minimum reflectance is determined and related to a refractive index and surface concentration of the bound substance at the sensing surface.

As regards a detection using surface plasmon resonance, this will be treated in more detail hereinafter.

Internal multiple-angle Brewster angle reflectometry, based on rotating optical means and a rotating prism for the variation of incident angle, has been shown to provide information to characterize the adsorption of proteins on a silica prism/solution interface; see Schaaf, P. et al. Langmuir, 3, 1131-1135 (1987).

Critical-angle refractometry has so far been used mainly to measure the concentration or density of solutions in process streams.

In a detection employing evanescent wave ellipsometry, the optical response related to the specific substance is measured as changes in the state of polarization of elliptically polarized light upon reflection, in that this state of polarization is related to a refractive index, thickness, and surface concentration of bound sample at the sensing surface. Multiple-angle evanescent wave ellipsometry, in the

form of using rotating optical means and a rotating prism for the variation of incident angle, and a phase-modulated ellipsometer, has been used for studying the polymer (polystyrene) concentration profile near a prism/liquid interface; see Kim, M.W., Macromolecules, 22, (1989) 2682-2685. Furthermore, total internal reflection ellipsometry in the form of stationary optical means at a single angle of incidence has been suggested for quantification of immunological reactions; see EP-A1-0 067 921 (1981), and EP-A1-0 278 577 (1988).

By a detection employing evanescent wave excitation fluorescence through an angle of incidence dependent TIRF, the intensity and wavelength of the radiation emitted from the either natively fluorescent or fluorescence labeled sample molecules within the sensing layer is measured. Total internal reflection fluorescence (TIRF) was suggested for a immunoassay already in 1974, in that a separate antigen-coated quartz slide was brought into optically contact to a quartz prism via a drop of index-matching solution and by using a Teflon O-ring sealed plexiglas cell, Kronick, M.M. et al., J. Immunol. Meth., 8, (1975) 235-240. Nowadays total internal reflection fluorescence is an established technique to examine interactions of macromolecules with solid surfaces, the surface being generally one side of the coupling prism; see Lok, B.K. et al., J. Colloid Interf. Sci., 91, (1983) 87-103. Furthermore, variable-angle total internal reflection fluorescence has been used to study adsorbed protein concentration profiles at a prism surface; see Reichert, W.M. et al., Applied Spectroscopy, 3, (1987) 503-508.

In a detection employing evanescent wave excitation scattered light, through an angle of incidence dependent evanescent wave penetration depth, the intensity of the radiation scattered within the sensing layer due to its interaction with the specific substance is measured. Scattered total

internal reflectance (STIR) has been suggested for utilization
in immunoassays, in that in a preferad embodiment colloidal
gold is used as a label for the solution phase immunologically
active component; see EP-A2-0 254 430 (1987).

As regards the use of surface plasmon resonance (SPR), in
somewhat simplified terms SPR may be said to be a technique
according to which changes in the refractive index of a
layer close to a thin free-electron metal film are detected
by way of consequential changes in the intensity of a
p-polarized light beam reflected from the metal film (see
for example Raether H, Physics of Thin Films, Academic
Press, N.Y., $\underline{9}$ (1977) 145.

In the first publication indicating the possibilities of SPR
technology in biochemical analysis, Liedberg B et al.,
Sensors and Actuators, $\underline{4}$ (1983) 299 have at first adsorbed a
monolayer of IgG to a silver surface and then adsorbed to
said monolayer a layer of anti-IgG, in order to then study
the effect in respect of the resultant change in the resonance
angle. EP 202 021 describes a biosensor employing movable
optical instrumentation for determining the angle - henceforth
called resonance angle - at which surface plasmon resonance
occurs. Such movable optical units are not suitable for
commercial-type instruments because (i) when readings of the
resonance angle are to be taken this will require manual
operations, and (ii) technical manufacturing tolerances in
the suspension mechanism of the movable optical system are
contributory to errors occurring in the measurements of the
resonance angle. EP 257 955 describes another optical system
which is scanned mechanically for determining the resonance
angle. GB 2 197 068 describes an optical sensor employing a
divergent beam of rays for irradiating the sensitized
surface, this latter being a metal film with receptors or
ligands which interact selectively with one or more bio-
molecules. The optical system is stationary, so the above-
mentioned drawbacks of movable optical systems are avoided.

A source of light is employed for irradiating a sensitized surface which is subjected to the action of a sample solution while another source of light is employed for irradiating another sensitized surface which is subjected to the action of a reference solution. The light sources and sensitized surfaces are arranged in such a way that the reflected divergent beams will strike a photodetector matrix. By means of alternate activations of each one of the two light sources the resonance angle obtained from each of the two sensitized surfaces can be measured with a good degree of precision, and the difference between the two resonance angles at each of the two sensitized surfaces will be a measure of the amount of the specific biomolecule bound on the sensitive layer. The disadvantage of this apparatus resides in the use of two individual sources of light - one for the reference solution, one for the sample solution - as this will tend to make the measuring result uncertain in view of the fact that the resonance angle is highly dependent on the spectral character of the light source. Another drawback of this known optical sensor resides in the positioning thereof directly on the prism of the optical system, and in having light directed to the sensitized surface via an immersion oil that has a suitable refractive index. Such a use of light-coupling oils will involve much practical inconvenience when the sensor unit, comprising a sensitized metal layer coated on a transparent plate, is to be replaced by a new sensor unit with a sensing surface having an affinity for a different specific biomolecule. The replacement operations will inevitably give rise to oil smears, and the prism has to be cleaned before a new sensing surface can be analyzed. Manipulation of the instrument will thus be a somewhat messy business. As to the actual structure of the analytical instrument, this is not disclosed in the aforesaid GB patent specification.

EP 226 470 describes an apparatus for microchemical analyses comprising two glass plates with a gel placed between them. The apparatus is one of the disposable type, to be used only once. One of the two glass plates serves as a platform on which the sample liquid is applied. Capillary force will then draw the sample liquid into the capillary cell that has been formed between the plates. A device of this type, the dimensions of which are about 3x1.5 cm, requires the use of tweezers or the like for handling. It is difficult to determine the volume of the liquid sample, and this device is therefore unsuitable for quantitative analyses.

EP-A1-0 305 109 (published after the priority date claimed in the present application) describes a SPR sensor system employing a focused (fan-shaped) light beam to illuminate the sensitive surface through a curved transparent block and via an index matching fluid. The beam enters the transparent block in a direction orthogonal to the tangent of the surface of the transparent block.

As was published by Kretschmann, E., Optics Communications, 26, (1978) 41-44, the problem of slow speed of operation relative to changes in reflectance and the insufficient precision in the resonance angle determination related with SPR procedures based on moveable mechanics, is solved by the use of a fan-shaped beam (equivalent to several beams incident upon the sensor surface over a range of angles) and of collection of the reflected beams (over a range of angles) by an array of angularly spaced detectors.

Furthermore, the transparent block described in EP-A1-0 305 109 may take the form of a hemicylinder creating a wedge-shaped beam, giving a line of a small illuminated area on the sensing surface. The hemicylindrical lens has the advantage that it can be used to perform several tests simultaneously on a single sample. To this end, the sensing surface takes

the form of a series of sensitive areas, each comprising a different antibody, with each separate area being monitored by its own detector in a detector array. The cylindrical focusing principle used to produce an identical angular range of light beams along a focused line for SPR of separate surface areas has been published by Benner, R.E. et al. Optics Communications 30 (1979) 145-149, and Swalen, JD et al. Am. J. Phys. 48 (1980) 669-672.

Further, a focusing lens in EP-A1-0 305 109 creates a substantially parallel-sided beam incident upon the detector, or a beam of at least of reduced divergence compared to the fan-shaped spread of light reflected from the sensing surface with the object to reduce stray light reflections in the detector array. The disadvantages of this apparatus resides primarily in the following: The approach to use a small illuminated area in relation to the sensitive layer for sensing, in order to reduce effects due to inevitable variations in a commercially produced metal film and coating of antibody. In fact, the surface concentration of bound sample molecules will in general also be non-uniform across the sensitive layer and strongly dependent on mass transport conditions. Thus, the small sensing area will be very sensitive to local variations of the sensing surface and its sample surface concentration resulting in a non-competitively low accuracy in the SPR response. Due to stray light arising from the coupling optics and at reflection in the sensor surface, it is possible to use the described optical system for monitoring beams from separate sensitive areas simultaneously by its own detector in an array only under the condition that the array can be conveniently placed close to the exit surface of the hemicylinder or attached to or deposited on that surface. This leads to limitations in the resolution of individual sensitive areas on the detector array, expensive optoelectronic constructions, complicated production process (detector alignment, optimization of collected angle span etc.). The use of an optical oil or

grease to ensure good optical coupling between the hemisphere and the sensor substrate (glass support plate or slide).

The approach of a disposable hemicylinder as optional technical solution for the change of sensor surface is unpractical due to the high costs of an adequate optical quality of this component and the critical optical alignment relative the light source.

As regard the use of internal reflection procedures, most of the publications that have come forth up to now thus describe laboratory equipment for singular determination of a specific substance at a time. Such equipments, however, are not suitable as practical commercial instruments; they are too complex and cumbersome in their construction for sample and liquid handling, detection, and evaluation, and the analysis procedures take quite a long time and moreover require highly skilled operators in order to obtain accurate results.

By the present invention a new analytical system is provided for at least one specifically sensing surface comprising the simultaneous detection of a plurality of specific interactions, in that it is adaptable for detection techniques based on cylindrically focused total internal reflection and internal Brewster angle reflectometry.

Thus, in one embodiment of the present invention, means for employing internal multiple-angle Brewster angle reflectometry on an exchangeable sensing surface for a new analytical system by use of a stationary optical system is provided.

By another embodiment of the present invention, means for employing multiple-angle evanescent ellipsometry on an exchangeable sensing surface is provided for a new analytical system by use of a stationary optical system.

In another embodiment of the present invention, a new analytical system is provided enabling variable-angle total internal reflection fluorescence on an exchangeable sensing surface by use of a stationary optical system.

In a still further embodiment of the present invention a new analytical system is provided enabling variable-angle scattered total internal reflectance (STIR) on an exchangeable sensing surface by use of a stationary optical system.

According to a preferred embodiment the present biosensor system uses a new mean-value procedure, utilizing an anamorphic lens system, for the detected reflectrometric, emitted or scattered light due to a specific interaction at a sensing surface that is optimized relative to the flow cell geometry, permitting inherently accurate and highly sensitive results to be obtained.

Furthermore, in a preferred embodiment the present invention provides a biosensor analytical system that eliminates the drawbacks related with more or less stationary sensing surfaces, generally being coated on expensive prisms or wave guides, by introducing a separate sensor unit that is semi-automatically exchangeable in the instrument. Such a sensor unit having at least two sensing surfaces, as well as a method for its functionalization, is the object of our copending PCT-application entitled "Sensor unit and its use in biosensor systems" (based upon Swedish patent application No. 8804074-6), the disclosure of which is incorporated by reference herein.

Moreover, the present biosensor system provides in a preferred embodiment thereof an automated integrated liquid handling block, micro-processor controlled valves therein, preferably membrane valves, enabling complex liquid handling tasks

comprising specific sequences of addition of various ligands,
macromolecules, and reactants for interaction at the sensing
unit.

The biosensor system of the present invention enables new
applications related to the field of characterizing bio-
molecules to be performed, e.g. as disclosed in our co-
pending PCT-application entitled "A method of characterizing
macromolecules" (based upon Swedish patent application
No. 8902043-2), the disclosure of which is incorporated by
reference herein; as well as in our aforesaid copending
PCT-application entitled "Sensor unit and its use in bio-
sensor systems". Such new applications made possible by the
present invention comprise:

-      qualitative as well as quantitative specific determin-
  ation of at least one biomolecule in a sample either
  simultaneously or sequentially,

-      qualitative as well as quantitative structural inform-
  ation of macromolecules through the detection of
  interactions between surface-exposed structural elements
  on the macromolecule and various ligands,

-      functional/structural information of macromolecules,

-      kinetic information about molecular interactions,

-      specific functionalization of at least one sensing
  surface on the sensor unit,

-      regeneration or changing of the specific function-
  alization of at least one sensing surface on the sensor
  unit.

In accordance with the above the optical biosensor system of
this invention comprises at least one, but preferably a

number of sensing surfaces or areas arranged in side-by-side relationship for being exposed to sample liquid passing over them. These sensing surfaces are analyzed by the above mentioned optical techniques based on a single source of light so that due to the use of one identical set of wave-lengths for all the sensing surfaces it is now possible to obtain a calibration, reference and evaluation process of such high analytical performance that this biosensor system becomes commercially useful. The system is also such that the sample liquid can be made to sweep over the sensing surfaces all at the same time or in a sequence. Measurement is to take place under controlled temperature conditions. At the time when the measurement is being performed the tempera-ture at all the sensing surfaces is to be the same and is to be kept constant during the measuring operation.

The sensing surface or surfaces of the sensor unit will lend themselves readily and simply to functionalization indivi-dually, for selective interaction with the desired bio-molecules; that is, it will be easy to bestow different affinity properties on these sensing surfaces.

The provision, in accordance with the above mentioned preferred embodiment, of a block unit for liquid handling using automated microprocessor-controlled sample insertion and sample guiding valves will permit precise and reproducible dispersion of the sample zone and accurately determined amounts and flow rates of sample solutions, reagent solutions or reference solutions.

The advantages of an integrated conduits liquid handling system situated in a permanent rigid and planar structure has been described by Ruzicka (Ruzicka, J., Analytical Chemistry, 55, 1041A (1983)). Hence, the rigidity of such a structure and the possibility to integrate and control sample injection ensures repeatability of dispersion of the

sample zone. Further, the small dimensions of the micro-conduits reduces sample reagent consumption to the microliter level. Furthermore, the versatile combination of engraved grooves as conduits within laminated parallel layers, interconnected by perpendicular channels, provides the means for integrating a number of highly controlled solution handling tasks. The technical limit on possible miniaturi-sation of such flow injection analysis systems has, however, been the availability of detectors suitable for sub-microliter detector volumes. The present invention comprises in one particular embodiment thereof a multi-analytical system enabling a detector volume of and below 60 nanoliter.

Thus the liquid handling block unit of the present biosensor system contains conduits or channels having one or more portions which when the measuring operation is to be performed are pressed against the sensor unit to thus form one or more flow cells, the arrangement being such that these flow cells can either be coupled in series or be made to receive each its own sample solution separately. A flow cell may contain a single sensing surface or alternatively a plurality of sensing surfaces; in this latter case, the sensing surfaces lie in a row in the longitudinal flow direction of the flow cell. The block unit for liquid handling is to be employed both when the sensing surfaces on the sensor unit are being functionalized and when analysis is carried out.

Furthermore, the optical biosensor system according to this invention provides for a stationary optical instrumentation, without any movable parts. Thereby the optical system may already in the course of its manufacture in the factory be given a fixed, "once for all" standard setting so that this setting does not need to be altered during the subsequent use of the system. In view of this fixed setting it is possible to have the optical system mounted inside a dust- and light-tight housing. Moreover no smeary oils need

to be employed for coupling the light to the replaceable
sensor unit. Instead a replaceable opto-interface may be
used for coupling light from the light source to the sensor
unit. Such an opto-interface is disclosed in our copending
PCT-application entitled "Optical interface means", the
disclosure of which is incorporated by reference herein.

As mentioned above the measurement accuracy may be still
further increased by establishing an optical and, respect-
ively, electronic mean value procedure as will be described
in detail below; this is so because the establishment of
such mean values will permit an accurate determination of
the quantitative or relative amount of specific biomolecules
that have adhered to the individual sensing surfaces.

With the features mentioned above, an optical biosensor-
based analytical system is obtained that permits fast
automated high-precision measurements of extremely low
concentrations, nanomolar and even down to picomolar, and
with a high response dynamics. In, for example, the SPR
biosensor system the resonance angle may be measured over a
wide range of angles.

The invention will now be described in greater detail below,
with reference to the attached drawings in which

      Fig. 1 is a schematic exploded view of an optical
      biosensor system according to the present invention,

      Fig. 1A is a partial cross-sectional view of Fig. 1
      showing a valve in its open position, and

      Fig. 1B is a corresponding view showing the valve in
      its closed condition,

Fig. 2 is a perspective view of the central part of the biosensor system,

Fig. 3 is a cross-sectional view of a sensor unit,

Fig. 4 is a top view of an irradiated sensor unit,

Fig. 5 is a perspective view of a carrier plate for the sensor unit,

Fig. 6 is a plan view of an opto-interface,

Fig. 7 is a sectional view of the opto-interface mechanically coupled to the sensor unit,

Fig. 8 is a schematic perspective view of the refractive characteristics of the anamorphic lens system, showing the creation of an optical mean value of the mass of biomolecules bound to the sensing surface, as seen along the width of the streak of light, this being achieved in that light rays in the plane P reflected at a certain specific angle irrespective of the point of reflection on the sensitized surface are imaged on a row of detector elements, and how in the plane S light rays from an individual sensing surface are imaged on a column of detector elements,

Fig. 9 is a schematic plan view in the S plane of the incident collimated beam of light, showing the creation of an electronic mean value of the mass of biomolecules bound to the sensing surface, as seen in the longitudinal direction of the beam of light,

Fig. 10 is a schematic perspective view of the flow cell, illustrating the flow conditions therein and the creation of an integrated optical mean value of the mass of bound biomolecules,

Fig. 11, top, shows a meridian section of the light source, a coupling prism and the anamorphic imaging system, and the first and second lens means; while at the bottom Fig. 11 shows a sagittal view of the same items,

Fig. 12 is a perspective view of a carrier and sensor unit in relation to a block unit for liquid handling,

Fig. 13 shows a plan view of the underside of the base plate in the Fig. 1 block unit for liquid handling,

Figs. 14A och 14B taken together show a plan view of the base plate according to another embodiment of a block unit for liquid handling,

Fig. 15 schematically shows the functions of the block unit for liquid handling according to Figs. 14A and 14B, and

Fig. 16 shows another embodiment of a flow cell in longitudinal or parallel mode in relation to light streak 5.

Fig. 1 shows the main components of the optical biosensor system according to this invention, in the form of an exploded view. The system comprises a source of light 1, first lens means 2 for directing a transversely extending convergent beam 3 toward a prism 4 whereby the beam is focused in the bottom surface of the prism to thus form a streak 5 of light. Rays of light reflected from the sensitized surfaces are imaged via an anamorphic lens system 6 on a two-dimensional photodetector device 7. The electric signals created by the photodetectors are processed in an evaluation device 8 in the form of a computer. By means of the prism and an opto-interface 9 light from streak 5 is directed to a sensor unit 10 which is to lie in contact with

a number of parallel, upwardly open portions 11A-D of flow
channels 14A to 14D respectively; only one of these, 14A, is
shown. The flow channels form part of a block unit 15 for
liquid handling, this unit being provided with schematically
indicated inlet connection means 16 and 96 and outlet
connection means 101 and 92.

Fig. 2 is a perspective view of the analytical apparatus,
generally designated 18, which is the heart of the optical
sensor system according to the invention. The apparatus
comprises a support 19 on which the block unit 15 for liquid
handling is mounted in a fixed position, and furthermore
comprises a housing 20 in which the light source 1, the
first lens means 2, the prism 4, the imaging optical unit 6
and the photodetector device 7 are accommodated in fixed
positions. An aperture 21 in the bottom portion of the
housing is to be covered by the opto-interface 9 which is
retained in its desired position by means of two guide pins
22, 23. The housing 20 is hinged to the support by means of
a system of articulated arms 24, 25 pivotally attached to
the housing and to the support respectively. The block unit
15 for liquid handling carries a number of holder means 26
for the sensor unit in its carrier plate. As shown in detail
in Fig. 12 the holder means are L-shaped angle pieces on top
of the upper face of the block unit for liquid handling,
thus forming guide means enabling the sensor unit to be slid
over said block unit. A thermostat means, not shown in
Fig. 2, surrounds prism 4. This thermostat means consists of
channels for liquid in a heat exchanger block which maintains
the metal frame around aperture 21 at a constant temperature.
A similar thermostat means 27 placed between the block unit
for liquid handling and the support is to maintain the
liquids in the channeling system of said block unit at the
same constant temperature as that stated above. The photo-
detector device is accommodated on a printed circuit card 28
mounted in a fixed position at one of the end faces of the

housing 20. Guide pins 22, 23 are received in openings 29,
30 provided in the block unit for liquid handling. A trans-
verse beam member 31 extending between the arms 24, 25
carries a projecting peg 32 for rotating the housing 20 back
and forth between two positions, viz., an analysis position
and a loading position. These will be described later in
greater detail. An electric motor 33 which is attached to
the support has an output shaft carrying a disk 34 excen-
trically, and the peg 32 is in contact with the periphery of
that disk 34. By activation of the electric motor so that
the excentrically mounted disk 34 rotates through a pre-
determined angle the housing is automatically swung into and
out of these analysis and loading positions without any
manual operation. A flat cable, shown schematically, goes
from the printed circuit card 28 to the evaluation device 8.

Fig. 3 shows the sensor unit in cross section. In our
copending Swedish patent application entitled "Sensor unit
and its use in biosensor systems" this sensor unit is
described in greater detail. The sensor unit comprises a
transparent plate 36 of glass, plastics or other transparent
material. On one of its faces the plate is provided with a
metal film 37 that has been applied by e.g. sputtering. A
dielectric film 38 is attached to the metal film. In the
preferred embodiment of the invention, the dielectric film
is a layer of dextran bound to the metal film. By means of a
coupling technique such as is known in biotechnology,
ligands are bound to the dextran film which serve to inter-
act with specific biomolecules present in the sample solution.
For attaching the ligands the sensor unit with its metal
film and the dextran layer thereon is contacted sealingly
with the upwardly open portions 11A-D of all the flow
channels 14A-D. By pumping a solution containing a specific
ligand L1 into one of the channels, 14A, another specific
ligand L2 into the second channel, 14B, a third specific
ligand L3 into the third channel, 14C etc., a corresponding

number of sensing surfaces may be produced on a single metal
film with each its own individual affinity for a (respective)
specific biomolecule. The ligands may for example be bifunc-
tional or polyfunctional molecules having one active portion
consisting of antidextran and another portion consisting of
an antigen to the antibody that is to be detected. Thanks to
this manner of functionalization, the optical biosensor
system of the invention may be tailored individually by the
user so as to be rendered useful for detection of just those
biomolecules in which he is interested. This functionalization
will permit inter alia qualitative analyses. The func-
tionalized sensor unit is introduced into the analytical
apparatus or functionalized in situ, whereupon the analysis
is carried out. When the analysis operation has been finished
the sensor unit is regenerated in that the sensing surfaces
are "washed" with a solution that will disrupt the coupling
between the ligands and the dextran film 38.

Fig. 4 is a top view of the sensor unit 10 of Fig. 3. The
sensor unit has been placed on top of the upwardly open
portions 11A-D, and the streak 5 of light arriving from lens
system 2 of light source 1 irradiates the reverse side of
metal film 37. As will be seen from the above description
regarding the activation of dextran film 38, sensing surfaces
39A-D are formed each corresponding to the length of the
open portions 11A-D (provided the sensor unit is attached to
the liquid handling block unit with the same orientation
both in the analysis phase and in the sensitation phase).
Light streak 5 extends transversely over the sensing surfaces
39A-D and has a width at least equal to the length of
portions 11A-D.

In Fig. 4, the metal surface 37 is presented on a magnified
scale. In actual practice the metal surface will be a square
of say an order of magnitude of about 10x10 mm, the dimensions
of the transparent plate then being somewhat larger, e.g.

11x11 mm. Such a construct is difficult to handle without
tweezers or other seizing means. But such procedures involving
the use of seizing tools are undesirable because they tend
to thwart high-precision measurement. What is proposed
instead, according to the present invention, is that each
sensor unit is mounted on a carrier plate which can easily
be seized between forefinger and thumb. A plan view of the
carrier plate is shown in Fig. 5; the plate comprises an
elongated sheet of plastic 42 with a grip area 43. An
opening 44 through the carrier plate has a flange 45 on
which the sensor unit will be resting. Clasps 46 projecting
laterally from the flange surface serve to retain the sensor
unit between the flange and clasps. The sheet 42 has two
guide holes 47, 48 through which the guide pins 22, 23 will
pass in the analysis position of the apparatus. When the
apparatus is in its loading position the guide pins are
retracted both from said guide holes 47, 48 and from the
openings 29, 30, the carrier plate being now free to be slid
into its position on the block unit for liquid handling or,
respectively, to be drawn off therefrom.

Sheet 42 also has control springs 49 for (i) facilitating
introduction of the sensor unit into the liquid handling
block unit and (ii) protecting the sensor unit from damage
or e.g. scratches in case the carrier plate is unintentionally
set down on a table or the like. It is recommendable to
accommodate the carrier plate and its sensor unit in a
housing which comprises top and bottom walls, two side walls
and one end wall. The interior faces of the top and bottom
walls are provided with pairs of opposite ledges extending
longitudinally of the housing. They serve to maintain a
clearance space between the carrier plate and the top and
bottom walls.

Fig. 6 shows a plan view of an opto-interface plate according
to the present invention. The plate is fastened on a metal

frame which has two projecting tongues 55, 56, said tongues being provided with one hole each, these holes 53, 54 extending all through the thickness of the tongues and serving to receive the guide pins 22, 23. The frame has two flanges 51, 52 against which a transparent plate 57 of glass or plastic has been applied. On one of its faces plate 57 is provided with a number of longitudinally extending parallel ridges 58 in side-by-side relationship. On its opposite face the plate has a corresponding number of parallel longitudinal ridges 59 lying opposite the ridges of the first-named plate face. The ridges are made of a transparent elastic material and spaced apart a distance corresponding to that between the upwardly open portions 11A-D of the flow channels. As will be seen more clearly from Fig. 7 the ridges 58, 59 have longitudinally extending stepped portions 60 at each side to thus form a structure having in cross section the configuration of a flight of stairs, the uppermost step or top platform of each of these stepped structures being capable of being pressed resiliently against the transparent plate 36 of the sensor unit and, respectively, the prism 4. This stepped configuration prevents air pockets from being formed between the interfaces contiguous to the prism and, respectively, plate 36 of the sensor unit. Fig. 7 shows the prism 4, the opto-interface plate and the sensor unit in the analysis position of the analytical apparatus 18. Ridges 58, 59 are spaced apart in such a manner that the distances between them correspond to the distances between the upwardly open portions 11A-D of the flow channels 14A-D.

This arrangement of the ridges 58, 59 on the opto-interface plate, the holes 53, 54 for the guide pins 22, 23, the holes 47, 48 on the carrier plate 42 of the sensor unit and the stationary mounting of the upwardly open portions 11A-D of the block unit for liquid handling ensure that the lower ridges 59 will serve as sources of light which lie directly above each of the corresponding channel portions 11A-D. No

scattered light from neighboring ridges 59 will interfere with the resonance angle determination for the individual sensing surfaces. In this manner it is possible to have a great number of these channel portions packed next to one another. As an example, it may be mentioned that up to 20 of such upwardly open channel portions can be packed together within a breadth of about 10 mm without any scattered light interfering with the measuring operation.

Figs. 8-11 illustrate the optical system in the analytic apparatus according to the invention. The basic principle of the anamorphic lens system 6 is shown in Fig. 8. The broad beam of light which is wedge-shaped or convergent strikes the illustrated sensitized surface, e.g. at sensing surface 39, at angles of incidence of from 62 to 78 degrees. Rays with all the intermediate (between 62 and 78°) angles of incidence are present in the beam. Consider only one incident plane P. All the rays incident with for example an angle of 62° are indicated by white arrows, are reflected on the sensitized surface and will be imaged on only one single photodetector 61A by the anamorphic lens system. Similarly, all the rays incident with an angle of 78° and indicated by black arrows will be imaged by the anamorphic lens system on one single photodetector 61G of the photodetector device 7. Light rays having incident angle values intermediate between 62 and 78 degrees will similarly strike those photodetectors which are situated between detectors 61A and 61G in the same photodetector column; in Fig. 8 this is conceived as being a vertical column.

Light source 1, e.g. a light emitting diode, emits a type of light that is substantially monochromatic in character (±30 nm), and furthermore is incoherent and has a center wavelength of an order of magnitude of about 650 to about 850 nm. The light passes through the first lens means 2 for forming a wedge-shaped convergent elongated beam which passes through a plane polarizer 63 indicated schematically

in Fig. 11 and then on toward the bottom face of prism 4. In that bottom face and on the underside of metal film 37 a streak of light is formed having a width which is adjustable by means of a cylindrical lens 64. The optical imaging system comprises the anamorphic lens system 6 the function of which has been described above. It contains inter alia a lens 66 for adjusting the degree of magnification of the resonance angle range along photodetector columns.

Light rays having a different plane of incidence parallel to the plane of incidence P will in a similar way be imaged on individual photodetectors belonging to other columns of the two-dimensional photodetector device. Every photodetector of a row thus corresponds to one specific angle of incidence. As against this, conditions are different in the case of the reflected light obtained from light that is reflected in an S plane, i.e. in planes differing from the plane of incidence P of the convergent beam. Such light will image a point on the sensitized surface as a real image element on detector pixel 61D. Thus to each detector column corresponds a respective part of the sensing surface as seen in the transverse direction of the conduit portion. Depending on the width of the channel, the surface dimensions of the individual photodetectors, and the spaces between them, a particular number of photodetector columns may be required for imaging the total width of the channel portion in question. In a preferred embodiment of the invention a reduced scale image of a large portion of the flow cell width is created on one single column of detectors.

Upon this introductory description of the anamorphic lens system will now follow a description of the mass uptake on a sensing surface, e.g. sensing surface 39D (see Fig. 9). The rate of flow is zero at the side walls of the channel, and then increases toward the center of the channel. The flow profile is shown schematically at 70 where arrow lengths correspond to the flow rates occurring. The channel width is

assumed to be about 300 µm. In order to avoid measuring
results becoming distorted by the outer channel regions
where no sample liquid is flowing the anamorphic lens system
is arranged so as to produce an image of only a narrow
portion, say of an order of magnitude of 50 % or more of the
width of the sensing surface, on a single column of photo-
detectors; e.g. a 150 µm central portion of a sensitized
surface whose total width is 300 µm is imaged on a column
pixel 61D having a width of 90 µm. This will electronically
produce a mean value of the mass uptake within this central
part of the sensing surface. Without such a creation of a
mean value of the amount of bound biomolecule as seen in the
transverse direction of the channel it would not be possible
to obtain such a high degree of reproducibility of the
response curve of any given sample concentration of the
biomolecule as is required in a commercial-type analytical
instrument - except perhaps by putting unrealistically high
demands on high reproducibility and control of (i) flow
profile in the sample liquid (thickness variation of diffusion
layer transversely of the channel), (ii) analysis position
of the instrument for optical units 2, 6 relatively to the
position of the flow channel, (iii) homogeneousness in
respect of ligand density and in respect of ligand access-
ibility within the dielectric film on the sensing surfaces.

Fig. 10 illustrates the flow conditions prevailing in the
channel portion below the sensing surface. The flow rate
profile of the sample liquid is illustrated by means of
arrows of different lengths. In the 90° bending region of
the flow channel a large amount of biomolecules may bind to
the sensing surface. Reference numeral 75 designates the
distribution of the amount of bound biomolecule. The amount
thus bound decreases downstream of the inlet. It will be
appreciated that if the resonance angle were to be determined
on the basis of only one section, indicated by arrows 76,
this would result in one particular value being obtained,
while if the resonance angle were determined on the basis of

the section indicated by arrows 77 this would result in another value therefor being obtained. In order to make sure that a reliable mean value of the resonance angle is obtained the width of light streak 5 is made to agree, according to the invention, with the length of the upwardly open channel portion. In this manner all the contributions from the amount of bound biomolecule will be included when the mean value of the resonance angle is being determined. An optically created mean value is thus obtained, as seen in the direction transversely of the path of the light beam.

Without such creating of a mean value of the amount of bound biomolecule as seen in the longitudinal direction of the channel it would not be possible to obtain such reproducibility of the response curve of any given sample concentration of the biomolecule, within a concentration range of interest for analysis purposes, as is required in a commercial-type analytical instrument - except perhaps by putting unrealistic- ally high demands on high reproducibility and control of (i) flow profile in the sample liquid in the longitudinal direction of the channel (thickness variation of diffusion layer in the direction of flow), (ii) sample dispersion in the direction of flow, (iii) homogeneousness in respect of ligand density and in respect of ligand accessibility within the dielectric film on the sensing surface, and (iv) the position of the focused light streak along the channel. With the creation of these mean values, it is possible to eliminate such deterioration of reproducibility as will otherwise occur due to variations in the position of the streak of light caused by variation in the thickness of the sensor plate. Maximum analytical sensitivity in the curve of response vs. concentration, and a minimum sample concentra- tion for the detection, are obtained for a light streak width and thus mean value creation covering the sensing surface at the riser duct that opens out into the flow cell, but with a reduced detectable maximum sample concentration.

The width of light streak 5 is adjustable by means of a
cylindrical lens 64 which is shown in Fig. 11. Alterna-
tively, light source 1 and lens means 2 are moved mutually
fixed along the optical axis. Fig. 11 illustrates the
anamorphic optical system in the form of both a meridian
section, uppermost part of the Figure, and a sagittal
section, lowermost part of the Figure.

The block unit for liquid handling will now be described.

Fig. 1 shows a flow channel 14A belonging to the upwardly
open portion 11A. The flow channels 14B-D belonging to the
respective upwardly open portions 11B-D have not been shown
here, for the sake of clarity. A first layer 80 of sealing
elastomer material, e.g. silicone rubber, has a number of
cuts or slits extending therethrough, corresponding to the
upwardly open portions 11A-D. The layer has been cast onto a
plateau 81 which is integral with a base plate 82. This
plate is preferably a solid member made of e.g. plastic,
metal, ceramics, silicon. A second layer 83 (Fig. 1A) of
elastomer material, for example silicone rubber, has been
applied by e.g. casting to the underside of base plate 82.
This layer 83 is provided with a system of flow channels
formed by casting. A third layer 84, preferably of the same
material as layer 83, has been cast onto a support plate 85.
of solid material, preferably the same as that of base plate
82.

It will now be understood that when in the analysis position
of the analytical apparatus the sensor unit 10 is pressed
against layer 80 by the opto-interface 9 the upwardly open
portions 11A-D in layer 80 will be sealed in liquid-tight
relationship against the sensor unit 10 and form four flow
cells. For the sake of simplicity, these flow cells too are
designated 11A-D.

There now follows a description of the principle according
to which a liquid sample is made to pass through flow cell
11A: By means of a pump (not shown) sample liquid is pumped
to inlet tube 16, passes through an inlet channel 87 past an
open valve 88 and then goes on through a primary channel 89
having a fixed and well-defined volume, until it reaches a
closed valve 90 whence it is directed into a waste channel
91 communicating via a connecting tube 92 with waste disposal
means 93.

Next, a valve (not shown) at the upstream end of waste
channel 91 is closed. At the same time valve 88 is closed.
Sample liquid in the primary volume is now ready to be
pumped into the flow cell 11A. This is done with the aid of
an eluent solution 94 which is pumped by a pump 95 through
an inlet tube 96 to an eluent conduit 97 ending in a valve
(not shown in Fig. 1, for the sake of clarity) which is now
opened, together with valve 90. Continued pumping of the
eluent solution will result in that the advancing eluent
solution will press forward against the primary volume of
the sample liquid and force it to advance upwardly through a
riser duct 98 in the plateau 81, thence into the flow cell
11A, and then down through a riser duct 99 in the plateau
and out through an exhaust duct 100 and an outlet tube 101;
then from this outlet tube at first the sample solution and
then the eluent solution will pass on to a second waste
disposal means 102 for sample and eluent solutions. When the
sample solution which has a predetermined volume is passing
along flow cell 11A the chemical action exerted by the
sample solution on the sensor unit is analyzed optically.

Valves 88, 90 and the other valves which have not been shown
in the drawing are identical in construction; therefore,
only valve 88 will be described. The valve comprises a
diaphragm 103 opposite a channel or through opening 104 in
the support plate 85. The valve diaphragm is an integral
part of layer 84. A valve seat 105 formed in layer 83 is an

integral part of that layer and has the shape of a projection
105. The portion of channel 104 leading to the valve is
filled with a short liquid column 106 of e.g. glycerol. Via
a compressed air supply line 107 and an electromagnetically
operated compressed-air valve 108 (shown only schematically)
the channel 104 communicates with a source 109 of compressed
air. The compressed-air valve 108 acts in response to
electric signals from the evaluation device 8 which is here
embodied in the form of a computer. Fig. 1A shows the valve
88 in its open position while Fig. 1B shows the same valve
in its closed position. Were it not for the liquid column
106 the compressed air would penetrate through 84 and pass
into the channel system in the form of undesirable air
bubbles.

Fig. 13 shows an embodiment of the channel system in a block
unit for liquid handling. Dotted lines 11A-D symbolize the
four flow cells each having their riser tube connection. The
small circular rings indicate valves each of which has a
construction as explained above. Eluent conduit 97 contains
valves 110, 111, 112 in the places where shown. Waste duct
91 has a valve 113. Immediately upstream of riser tubes
98A-D there are valves 114A-D, simply designated by the
common numeral 114 in Fig. 13, for the sake of clarity.
Immediately downstream of riser tubes 99A-D there are valves
115A-D; here again the valves are symbolized by only one
common numeral, 115. A second waste duct, designated 116,
has valves 117A-D in the places where shown; their one
common numeral is 117. Moreover the outlet tube 100 has a
group of valves 118A-D corresponding to valves 117. Two
different eluant solutions may be introduced into the system
through conduits 119, 120 leading to connecting tubes 121
for a mixing chamber (not shown) the outlet of which communi-
cates with eluent conduit 97. After the primary volume 89
has been filled in, the sample liquid can be directed to any
one of the flow cells 11A-D with the aid of the eluent
liquid, the valves 117 and 118 being maintained open and

closed in appropriate combinations. For instance, if the
sample solution is to be passed on to flow cell 11C, then
valves 114A, B and D are kept closed, 110 closed, 117A and B
open, 117C closed, 111, 118A and B closed, and 118C and D
open. 115A, B and D should also be closed.

An especially advantageous feature of the liquid handling
block unit according to Fig. 13 is that flow cells 11A-D can
be coupled in series with each other. In that case one and
the same sample liquid will pass through the flow cells one
after the other. The number of such serially arranged flow
cells may be 2, 3 or 4 depending on how the valves are set.
For example, if all the four flow cells are to be coupled in
series the following valves are maintained in the closed
position when the eluent solution is forcing the sample
solution out of the primary volume 89: Valves 110, 111, 113,
117A, 117C, 118B, 118D, 88; while the following are maintained
in the open position: Valves 114A-D, 115A-D, 112, 90, 118A,
118C, 117B, 117D. The sample liquid and eluent solution run
off to the waste disposal means via duct 116.

Another embodiment of the block unit for liquid handling is
shown in Figs. 14A, B and 15. This embodiment differs from
that of Fig. 13 in that a secondary volume for liquid
samples has been added and the liquid channel system has
been duplicated. The secondary volume, which has an exactly
defined volume, preferably different from that of the
primary volume, can be coupled in series with the primary
volume if and as desired. In this manner it thus becomes
possible to analyze two sample liquid volumes which differ
inter se and which both are well-defined. The duplicate
channel system is a time-saving feature when analyses are
carried out - for while the sample liquid of one channel
system is being pumped into the flow cells for analysis the
other channel system may at the same time be cleaned and
filled with fresh sample liquid; so that when analysis of

the sample liquid of the first channel system has been
finalized the sample liquid of the second channel system is
immediately ready and available for analysis.

The small rings in Figs. 14 and 15 designate valves 201,
202, 203, 204, 205, 206, 207, 208A-B, 209A-C, 210A-B, 211,
212A-B, 214, 215. Furthermore, riser ducts are provided
which are to be connected - via e.g. a block (not shown)
having flexible tube connections and planar sealings around
the riser duct ends on its underside - to the pump 96, to
the pump (not shown) for sample liquid, to the sample
solution waste disposal means 93, to the waste disposal
means from the two channel systems and to the waste disposal
means for sample solution plus eluent solution as shown to
the right in the Figure, 101.

Valves having the same numeral in Fig. 15 are all controlled
by one common compressed-air valve. Thus for instance, when
valves 208 are pressurized, all the valves 208A, 208B, 208C
and 208D are pressurized simultaneously.

If sample solution is to be pumped into the lowermost
channel system of Fig. 15 then valves 209B, 214, 215, 209D
and 213 are maintained in closed positions so that the
sample liquid can pass through the open valves 208D to the
primary volume 122 and thence to the secondary volume,
designated by 123. If this secondary volume, too, is to be
filled valve 213 is kept closed. Valves 212A and B are kept
open, 209C closed, 208C open, the sample liquid being
discharged via the waste disposal duct 124 of the lowermost
channel system. When sample liquid in the primary and
secondary volumes is to be analyzed valves 208 are closed,
valves 209 and 212 are opened, 213 is closed; and eluent
solution is now pumped through the lower eluent channel 125
to force sample solution in the primary and secondary
volumes through a desired flow cell 11A-D. Valves 201-207

serve as switch mechanisms for directing sample liquid to
the desired flow cell. Sample and eluent solutions will then
be discharged through an exhaust duct 126 which is common to
both channel systems.

Correspondingly the uppermost channel system has an eluent
channel 127 and a waste conduit 128, a primary volume 132
and a secondary volume 133.

Valves 208B and D, valves 209B and D, and valves 214, 215
serve as means for selecting the desired channel system,
i.e. causing sample liquid to be pumped either into the
lowermost channel system 135 or into the uppermost channel
system 136. The sample liquid is pumped in through a common
inlet channel 129.

In order to avoid sample carry-over, the T-section of the
channel 129 can be washed. When the channel system 136 is
washed, a small volume of the washing liquid is released
through the waste disposal channel 130 by opening the valve
215 for a short time. When the channel system 135 is washed,
a small volume of the washing liquid is released through the
waste disposal channel 131 by opening the valve 214 for a
short time.

Although not shown in Figs. 14A, B and 15 the block unit for
liquid handling may be provided with valves corresponding to
valves 115, 18 in Fig. 13 to thus enable the flow cells to
be coupled in series with one another.

By means of separating the valve functions so that every
valve, i.e. also each of the valves 208A, 208B, 208C and
208D, is actuated by a separate compressed-air valve of its
own, possibilities are provided to pump either the primary
volume or the secondary volume, or the primary plus secondary
volume, through a desired flow cell.

As may be seen from Figs. 14A and 14B the inlet channel 129
for sample liquid and the inlet channels 125, 127 for eluent
solution all have a considerable length. This is so because
the temperature is to be kept constant and equal in both of
these solutions with the aid of the thermostat means 27
(shown in Fig. 2) which is situated below these channel
portions and serves as a kind of a heat exchanger. The
thermostat means 27 and the thermostat means in housing 2
are both of the liquid-operated type, their liquid flows
emanating preferably from one common liquid bath which is
maintained under strict temperature control.

In the above-described embodiments of the invention a
plurality of flow cells - up to 20 - are arranged side by
side and are irradiated simultaneously by a streak of light
extending transversely over the flow cells and their sensing
surfaces.

According to the invention it is also possible to employ
just one flow cell the longitudinal direction of which is
parallel to the streak of light, with a number of mutually
spaced apart sensing surfaces which are arranged within the
flow cell, are irradiated simultaneously by the light streak
and are each functionalized in the manner described above.
After functionalization in an apparatus with flow channels
similar to that shown in Fig. 1 the sensing surfaces 40A-D
are oriented as shown in Fig. 16. As will be seen from this
Figure, the block unit for liquid handling 140 which is then
used in the analysis phase may have just one single flow
cell 141 instead of the four cells shown earlier. The chief
advantage of such an arrangement resides in obtaining a
short and well-defined interface between the sample liquid
and eluent solution, as compared to the interface formed
when the flow cells are coupled in series and the sample
liquid travels along a meander-type path through the serial
cells; in this case the interface tends to become elongated
at each bend of 180°.

From the above description of the block unit for liquid
handling it will be evident that the number of primary
volumes may be increased and that such additional volumes
may each be given any size as desired.

According to still another embodiment of the channel system
in a liquid handling block unit of the type having a
plurality of flow cells in side-by-side relationship, all
the flow cells are simultaneously fed each with one liquid
of its own. In this manner the liquids can be analyzed
simultaneously in the analytical apparatus. The liquids may
be sample solutions of different types or of the same type.
They may also be a number of sample solutions plus one or a
plurality of reference solutions. This manner of parallelly
analyzing a plurality of liquids is conducive to shortening
the time required for analysis. Still another variant form
of the invention resides in (i) providing a plurality of
sensing surfaces in each of the flow cells lying next to
each other side by side, and (ii) feeding all the flow cells
simultaneously each with one liquid of its own. In this case
the time required for analysis is shortened to a still
greater extent, and a possibility is provided of quali-
tatively analyzing a greater number of substances simul-
taneously.

According to an alternative embodiment the layer 80 may be a
plate of silicon into which slits extending therethrough and
corresponding to the upwardly open channel portions 11A-D
have been etched by means of a technique such as is known
within the field of micromechanics.

Knowing the cross sectional area of the flow channel and
also the volume capacity of the pump (e.g. in µl per unit
time) one is enabled to carry out a time-controlled pumping
operation whereby a liquid volume chosen as desired is
pumped along the sensing surfaces. Now looking at Fig. 15,
suppose primary volume 122 has a capacity of 5 µl and

secondary volume 123 has a capacity of 45 µl: By means of
opening valve 213 and closing valves 212A-B, alternatively
opening valve 211 and close valves 210A-B, after a pre-
determined period of time corresponding to the desired
liquid volume to be analyzed it is thus possible to feed any
desired volume of between 5 and 50 µl along a sensing
surface.

Solenoid-activated valves may be employed instead of the
pneumatically activated valves. Such a solenoid-activated
valve may consist, according to the invention, of a wire of
superelastic alloy attached to the solenoid armature. The
wire extends through holes in the support plate into contact
with the layer 84. When the solenoid is activated the end of
the wire will push up the diaphragm 84 of the valve against
the valve seat 105 in a similar way as shown in Fig. 1B.
Typically the valve openings in the support plate 88 have a
diameter of 0.5 mm; consequently the superelastic alloy
wires should have a diameter of an order of magnitude of
0.4 mm. Superelasticity in this case means that the wires
may be formed with sharp bends but nevertheless remain
stiff.

Fig. 12 shows the region around plateau 81 of the block unit
for liquid handling. As will be seen, there are a number of
L-shaped angle pieces 101-105 on each side of the plateau
for retaining carrier plate 42 in its position over the
sheet 80.

It will be appreciated that when the analytical apparatus 18
is in its loading position, the housing being raised and the
guide pins being retracted from openings 29, 30, a carrier
plate 42 can be slid in between the L-shaped angle pieces
100-105. Then when the electric motor rotates and lowers its
head the prism 4 in aperture 21 of housing 20 will be
pressed against the opto-interface plate and thus also
sensor unit 10 against layer 80 in a liquid-tight manner due

to the weight of the housing. When the metal film 37 of the
sensor unit 10 and the dielectric film 38 on metal film 37
are sealingly pressed against layer 80, flow cells will be
created as formed by the aforesaid upwardly open portions
11A-D.

For initial calibration of the analytical apparatus, salt
solutions each having a known refractive index are pumped
one after the other past each of the sensing surfaces. The
electric signals from the detectors of the photodetector
columns corresponding to each of the sensing surfaces are
stored in a memory, separately for each one of the salt
solutions. By curve fitting of the response values from each
of the photodetectors all the photodetector members of the
analytical apparatus are calibrated so that a linear
relationship is established between the salt concentrations
and the measured refractive indices. Next, the sample
solution is pumped through a flow channel 14 which serves as
a reference channel; its sensing surface has no affinity for
biomolecules at this particular time when the calibration is
carried out. The electric signals obtained from the photo-
detector rows corresponding to the resonance angle of the
sample solution are stored in another location of the
memory. The purpose of this calibration is to determine the
refractive index of the sample solution. The stored signal
corresponding to that index is then corrected from the
signals obtained when the sample solution is pumped through
a flow channel with an activated sensing surface in order to
determine a difference in refractive index due to the
presence of the target biomolecule in the sample solution.
The same reference channel may also be used for temperature
monitoring of the analytical procedure; in case unnatural
temperature variations are detected the pumps are stopped
and analysis is discontinued.

By coating at least one of the sealing areas of layer 80
between the flow channels with a dielectricum of a suitable

refractive index in order to gain SPR response, the tempera-
ture at layer 80 can be monitored. The refractive index of
most organic materials is sensitive to the temperature, and
the dielectricum should have a known temperature dependence
of its refractive index. Light beams reflected at the metal
film 37 at areas thereof opposite to the film areas pressed
in close optical contact with the liquid sealing areas of
the dielectricum will be imaged by the anamorphic lens
system 6 onto corresponding columns of detector elements,
e.g. situated between columns of detector elements corre-
sponding to flow channels. The electrical signal representing
the resonance angle as measured at the sealing areas between
the flow channels will represent an actual temperature value
at the dielectric layer. By guidance from the measured
difference between the corrected signal and the actual
value, the thermostat system can be controlled towards the
temperature set point selected. The measured electric signal
may also , as an alternative, or complement, to the above
described method, be used to correct for the baseline drift
caused by temperature disturbances due to the thermostat
means not being positioned in the flow cell.

The optical biosensor system instrument schematically
described for a surface plasmon resonance detection in
figures 1 and 8, may also be adapted for detection using
critical angle refractometry, multiple angle evanescent wave
ellipsometry, and Brewster angle reflectometry, in that the
first lens means 62 and the second lens means 65 are generally
similar for these various detection principles. It should be
noted, however, that the addition of specific optical
components are required for the employment of ellipsometry
in the optical biosensor system.

Additionally, the optical biosensor system instrument
described in figures 1 and 8, may be arranged for employing
variable angle total internal reflection fluorescence,
variable angle total internal reflection phosphorescence,

variable angle total internal reflection light scattering. More specifically, the second lens means 65 may be positioned for detection of in-line fluorescence or phosphorescence, or as indicated in figure 1, by the second lens means 165 and photodetectors 161 for detection of right-angle fluorescence or phosphorescence, or of right angle light scattering.

The above-described embodiments of the invention may be modified and varied in many ways within the scope of the inventive concept.

CLAIMS

1. An optical biosensor system using internal reflection versus angle of incidence determination for detecting a specific biomolecule, for example an antigen, and comprising:

    -   a sensor unit (10) in the form of a transparent plate (36), at least a portion of one of the faces of said plate (36) either having a dielectric film adhered thereto, or being primarily coated with a metal film (37) to which has been adhered a dielectric film (38), said dielectric film having an affinity for the biomolecule at the time when the measurement is carried out,

    -   a light source (1) with first lens means (62, 64) for directing a beam of light against the interface of the transparent plate (36) and its coating of said film or films and which light may produce at least one evanescent wave in a sensing surface of said film or films when a sample solution contacts the dielectric film on the transparent plate,

    -   optical imaging instrumentation with second lens means (65) by which light reflected from the sensitized surface of the transparent plate (36) is imaged on a photodetector device, or

    -   optical imaging instrumentation with second lens and optical filter means (165) by which light originating from the sample, through either evanescent wave stimulated fluorescence (TIRF), phosphorence, or light originating from scattering at the sensing surface, is imaged on a photodetector device,

-    an evaluation unit (8) for determining the angle
     for minimum reflectance, and/or the state of
     polarization, and/or the collected light intensity,
     characterized in

-    that at least one, and preferably two or more
     sensing surface (39A-D) arranged in side-by-side
     relationship, are defined on the sensor unit,

-    that when measurement is to be carried out the
     sensing surface or surfaces (39A-D; 40A-D) in
     order to form an elongated flow cell are to be
     pressed over an open portion (11A-D) of at least
     one channel (14A-D) for the sample solution which
     is to be analyzed and affects the angle and/or
     polarization state for minimum reflectance, and/or
     the collected light intensity, at which the
     evanescent wave interacts with the sample,

-    that the first lens means (62) form a convergent
     beam of light focused in wedge-shape fashion into
     forming a streak of light extending transversely
     over all the sensing surface or surfaces,

-    that the photodetector device (61) is a two-
     dimensional matrix of individual photodetectors,

-    that the second lens means (65) of the optical
     imaging instrumentation is an anamorphic lens
     system for imaging rays of reflected light from
     the sensing surface or surfaces on each its own
     column (61A-G) of photodetectors, so that to each
     sensing surface will correspond to one particular
     set of columns of photodetectors, or

-    that the second lens means (165) of the optical
     imaging instrumentation is a lens system, which

may be anamorphic, for imaging rays of light
emitted through fluorescence or phosphorence from
the sample or light originating from scattering at
the sensing surface, on each its own column
(61A-G) of photodetectors, so that to each sensing
surface will correspond one particular set of
columns of photodetectors,

- and that the evaluation unit (8) is arranged to
determine the angle for minimum reflectance,
and/or the state of polarization, and/or the
collected light intensity, for each of the sensing
surfaces.

2. An optical sensor system according to claim 1, charac-
terized by

- a support (19) in which the open portion or
portions (11A-D) of the at least one channel
(14A-D) is (are) arranged in a fixed position(s),

- a housing (20) in which the light source, the
optical imaging instrumentation, the photodetector
device and a coupling prism (4) are arranged in
fixed positions,

- an aperture (21) provided in the housing below the
coupling prism and covered by an opto-interface
plate (9) for coupling incident and reflected
light and/or scattered or emitted light from
sample, respectively, to said sensor unit and
optical imaging instrumentation,

- and holder means (100-104) by which the sensor
unit is detachably attached below the aperture,

- the housing being meant to be placed on top of the sensor unit for pressing the opto-interface plate against the other face of the sensor unit.

3. An optical sensor system according to claim 2, characterized in that the housing (20) is hinged to the support (19) by means of a system of articulated arms (24-25) extending from each lateral face of the housing and the support respectively.

4. An optical sensor system according to claim 2 or 3, characterized by thermostat means (27) provided in the housing and at the at least one channel for the purpose of thermostatically controlling each sensing surface and channel respectively.

5. An optical system according to any one of claims 1-4, characterized in that at least two sensing surfaces (39A-D) are arranged in side-by-side relationship on the sensor unit (10).

6. An optical sensor system according to any one of claims 1-5, characterized in that the plate (36) of the sensor unit is of glass.

7. An optical sensor system according to any one of claims 1-6, characterized in that the dielectric film (38) comprises a dextran layer.

8. An optical sensor system according to any one of claims 1-7, characterized in that a plurality of channels (14A-D) are arranged in parallel side-by-side relationship, that each channel has an upwardly open portion (11A-D), that these upwardly open portions together are to be covered by the sensor unit in the

stage when measurement is performed, and that in the said measuring stage each sensing surface is situated above each its own upwardly open portion.

9.   An optical sensor system according to claim 8, characterized in that the upwardly open channel portions lying in side-by-side relationship are slits (11A-D) extending through a first layer (80) of elastomer material or silicon.

10.   An optical sensor system according to claim 9, characterized in that the first layer is made of rubber material, preferably silicone rubber.

11.   An optical sensor system according to any one of claims 8-10, characterized in that the channels (14A-D) are formed in a liquid handling block unit (15) of a solid material such as plastics, preferably polystyrene, metal or ceramics.

12.   An optical sensor system according to any one of claims 1-11, characterized in that the channels (14A-D) can be coupled in series with each other.

13.   An optical sensor system according to claim 11 or 12, characterized in that the liquid handling block unit comprises

-      a planar support plate (85) carrying a second elastomer layer (84) on its upper face,

-      a base plate (82) positioned on top of the second elastomer layer and provided with

-      a third elastomer layer (83) positioned on the underside of the base plate,

- a first number of riser ducts (98) which correspond to the number of upwardly open channel portions (11A-D), extend through the base plate, and communicate with one end of each their respective open channel portion,

- a second number of riser ducts (99) which correspond to the number of upwardly open channels and communicate with the opposite end of each their respective open channel portion,

- a pattern of channels formed in the third elastomer layer and having

- at least one primary volume (89; 122) for sample liquid, said primary volume having an exactly determined volume,

- at least one inlet channel (87; 129) for sample liquid,

- at least one inlet channel (97; 125, 127) for eluent liquid,

- an outlet channel (100; 126) common to said sample and eluent liquids,

- valve means (103) accommodated on the support plate for creating desired communications with the channels of the channel system.

14. An optical sensor system according to claim 13, characterized in that the channel system comprises a secondary volume (123) for sample liquid, said secondary volume having an exactly determined volume which preferably differs from that of the primary volume.

15.   An optical sensor system according to claim 14, charac-
      terized in that the channel systems are duplicated so
      that one system can be filled while sample liquid of
      the other system is being analyzed.

16.   An optical sensor system according to claim 13 or 14,
      characterized in that the valves are pneumatic valves
      formed by through openings (104) in the support plate
      (88), by the second elastomer layer (84) above these
      openings, and by valve seats in the form of projections
      (105) from the third elastomer layer (83) of the base
      plate (82).

17.   An optical sensor system according to claim 13 or 14,
      characterized in that the valves are electromagnetic
      valves having a wire attached to their armature, said
      wire being made of superelastic material and extending
      through the opening in the support plate to act upon
      the second elastomer layer.

18.   An optical sensor system according to one or more of
      the preceding claims, characterized in that the upwardly
      open portion (11A-D) has a length of about 800 µm, a
      width of about 300 µm, and a height of about 30 µm.

19.   An optical sensor system according to claim 2, charac-
      terized by

      -      a carrier plate (42) for the sensor unit,

      -      an opening (44) in the carrier plate such as to
             snugly accommodate the sensor unit, with said unit
             resting on flange means (45) at the lower end of
             said opening, and

      -      a grip portion (43) on the carrier plate, to be
             seized between thumb and forefinger.

20. An optical sensor system according to claim 19, charac-
terized by a separate storage housing for each carrier
plate with the sensor unit mounted thereon, said
housing comprising top and bottom walls, two side walls
and an end wall.

21. An optical sensor system according to any one of
claims 1-20, characterized by the anamorphic second
lens system (6) being arranged for

-    imaging reflected rays of light from surface
     elements in the longitudinal direction of the
     streak of light, in the form of real image elements
     corresponding to rows in columns of photodetectors
     where each column corresponds to each its own part
     of the sensing surface in the direction of the
     streak of light,

-    imaging reflected rays of light from surface
     elements transversely of the longitudinal direction
     of the streak of light, onto pixels situated along
     columns of photodetectors, in that mutually
     parallel incident rays lying in the plane of
     incidence and aligned transversely of the longi-
     tudinal direction of the streak of light are
     focused toward a single photodetector in a column,
     so that to each angle of incidence of the incident
     light corresponds each its photodetector (61A-G)
     in a column, i.e. each detector in a column
     corresponds to one particular angle of incidence.

22. An optical sensor system according to any one of claims
1-21, characterized in that said internal reflectance
versus angle of incidence determination is based upon
surface plasmon resonance.

23.   An optical sensor system according to claim 21 or 22, characterized in that the convergent beam of light strikes the reverse side of the metal film (37) with angles of incidence of between 62 and 78 degrees.

24.   An optical sensor system according to any one of claims 1-20, characterized by

-     the second lens system (165), which may be anamorphic, being arranged for collecting rays of light emitted from sample or rays of light scattered from surface elements in the longitudinal direction of the streak of light, onto columns of photodetectors where each column corresponds to each its own part of the sensing surface in the direction of the streak of light,

-     collecting rays of light from surface elements transversely of the longitudinal direction of the streak of light, onto pixels situated along columns of photodetectors, in that mutually parallel incident rays lying in the plane of incidence and aligned transversely of the longitudinal direction of the streak of light are focused toward a single photodetector in a column, so that to each angle of incidence of the incident light corresponds to each its photodetector (61A-G) in a column, i.e. each detector in a column corresponds to one particular angle of incidence.

25.   An optical sensor system according to any one of claims 1-24, characterized in that the width of the streak of light irradiating the sensing surfaces is substantially equal to the extent of the sensing surfaces transversely of the longitudinal direction of the streak of light, thus resulting in the optical

creation of an integrated mean value of the mass of biomolecules binding to the dielectric film to thus permit a reproducible quantitative analysis or alternatively a reproducible analysis of the relative amount of the particular biomolecule contemplated.

26. An optical sensor system according to claim 25, characterized in that the extent of a sensing surface as seen in the longitudinal direction of the streak of light is imaged in reduced size on a single photodetector, thus resulting in the electronic creation of an integrated mean value of the mass of biomolecules binding to the dielectric film as seen in the longitudinal direction of the streak of light.

27. An optical sensor system according to claim 26, characterized in that the anamorphic lens system is arranged for size reduction of an image element in the S planes with a linear degree of magnification m = 0.56 when said extent is of the order of magnitude of about 300 μm and a photodetector has dimensions of about 90x90 μm.

28. An optical sensor system according to any one of claims 1-27, characterized in that the first lens means (62) of the light source (1) comprise a lens member (64) for varying the width of the streak of light irradiating the sensing surfaces.

29. An optical sensor system according to claim 2, characterized in that the coupling prism (4) is in the form of a truncated straight hemicylinder placed over the aperture in the bottom wall of the housing.

30. An optical sensor system according to claim 23, charac-
terized in that the light source (1) emits substan-
tially monochromatic and incoherent light, e.g. being a
light emitting diode in combination with an inter-
ference filter.

31. An optical sensor system according to claim 25, charac-
terized in that the light source (1) emits substantially
monochromatic and coherent light, e.g. by use of a
semiconductor diode laser, a dye laser, or a gas laser.

32. An optical sensor system according to claim 2, charac-
terized by positioning means (22, 23, 29, 30) for
setting the positions of the housing (20), the optical
sensor (10) and the support (19) relatively to one
another.

33. An optical sensor system according to claim 2, charac-
terized in that the opto-interface plate (9) comprises
a transparent plate (57) of e.g. glass carrying on one
of its faces longitudinally extending parallel ridges
(58) aligned with longitudinally extending parallel
ridges (59) on the opposite face of the plate, these
ridges being (i) made of an elastic transparent material,
(ii) spaced apart with distances inter se corresponding
to the distances between the sensing surfaces (39A-D),
(iii) of a length at least sufficient to have the
entire cross section of the convergent beam of light
coupled to the sensor unit, and (iv) intended for being
pressed against the transparent plate of the sensor
unit directly opposite the sensing surfaces, to thus
direct the light into the sensor unit (10).

34. An optical sensor system according to claim 33, charac-
terized in that each ridge (58, 59) has a number of
longitudinally extending stepped portions (60) at each
side to thus form a structure having in cross section

the configuration of a flight of stairs, the uppermost steps thereof being capable of being pressed resiliently against the sensor unit and, respectively, the prism (4) without formation of enclosed air pockets.

35. An optical sensor system according to claim 24, characterized in that the evaluation unit is arranged in such a manner that when the mass of bound specific biomolecules is calculated this calculation will include light emitted from sample or scattered from sensing surface from only a partial band of the width of each sensing surface, said partial band extending substantially along the entire length of the sensing surface and being positioned centrally therein, this operation being effectuated by evaluation of the electric signals obtained from the photodetectors in that column which corresponds to a corresponding partial band on each of the sensing surfaces.

36. An optical sensor system according to claim 26 or 27, characterized in that the evaluation unit is arranged in such a manner that when the mass of bound specific biomolecules is calculated this calculation will include reflected light from only a narrow band, preferably about 50 %, of the width of each sensing surface, said narrow band extending substantially along the entire length of the sensing surface and being positioned centrally therein, this operation being effectuated by evaluation of the electric signals obtained from the photodetectors in that column which corresponds to a corresponding narrow band on each of the sensing surfaces.

37. An optical sensor system according to claim 36, characterized in that the evaluation unit is arranged for calculating parameters for a surface plasmon resonance curve for a determination comprising a reflectance

minimum and a resonance angle and the displacement thereof for each of the flow cells, by means of curve fitting of the response values obtained from column detector members corresponding to the resonance angle, i.e. the reflectance minimum.

38. An optical sensor system according to claim 2, characterized in that the coupling prism (4) is in the form of a plane sided prism placed over the aperture in the bottom wall of the housing, the refractive properties of said plane sided prism having been taken into account when designing the first lens means (62) for forming a convergent beam of light focused in a wedge-shaped fashion into forming a streak of light extending transversely over all the sensitized areas.

39. An optical sensor system according to any one of claims 1-38, characterized in that the open channel portion situated at the sensing surface has the form of a wall-jet cell, for which the jet flow is directed against the centre of such a circular sensing surface.

40. A method of calibrating an optical sensor device according to claim 1, characterized in

   - that calibration solutions such as salt solutions and/or suitable organic solutions without affinity to the sensing layer, each with a known refractive index, are pumped one after the other through a flow cell,

   - that the electric signals from the detectors of the photodetector rows corresponding to each one of the sensing surfaces are stored in a memory, for each one of the calibration solutions, so that a known relationship is obtained between the resonance angle and refractive index in the dielectric film,

- and that the responses from each of the photo-
  detector members are calibrated.

41. A method according to claim 40, characterized in

   - that the sample solution is passed over a separate
     sensing surface that has no affinity for any
     biomolecule but is situated on the metal film,

   - that the electric signals obtained from the
     photodetector rows corresponding to the separate,
     non-affinity sensing surface are stored in another
     location of the memory,

   - and that the stored signals are corrected from the
     signals obtained when the sample solution is made
     to pass along those sensing surfaces that do
     possess affinity properties, to thus determine a
     difference between the refractive indices due to
     the presence of the particular biomolecule for
     which the sensing surface possesses affinity
     properties.

42. Method of correcting for baseline drift in SPR analysis
     procedures using the optical biosensor system according
     to claim 1, characterized in

   - that a predetermined temperature set point is
     established for the sample solution,

   - that for creating an electric signal representing
     an actual temperature value of the sample solution,
     electric signals are employed which represent the
     resonance angle as measured in a flow cell the
     sensing surface of which has no affinity for the
     specific biomolecule and through which a liquid is
     fed which is conveyed through the liquid flow
     handling unit,

- that the signals from the sample solution are corrected in respect of the temperature disturbance caused by the fact that the thermostat means is not positioned in the flow cell, and

- that by guidance from the measured difference between the corrected signal and the actual value the thermostat means is controlled towards the temperature set point.

43. A method of measuring the actual feedback value of the temperature at the flow channels for regulation of the thermostat system by SPR analysis procedures using the optical biosensor system according to claim 1, characterized in

- that for creating an electrical signal representing an actual temperature value of the sample solution, electric signals are employed which represent the resonance angle as measured at a flow cell the sensing surface of which is in contact with at least one of the sealing areas (80) adjacent or between the flow channel(s), which sealing area is coated with a dielectricum of suitable material and refractive index properties in order to obtain a SPR response from said area, said dielectricum, having a known temperature dependence of its refractive index, light beams reflected at areas of the metal film (37) opposite to areas of the film pressed in close optical contact with the liquid sealing areas of said dielectricum being imaged by the anamorphic lens system (6) onto corresponding columns of detector elements, and

- that by guidance from the measured difference between the corrected signal and the actual value the thermostat means is controlled towards the temperature set point.

FIG.1

Data communication

FIG.1A

Valve open

Valve closed

FIG.1B

FIG. 2

FIG. 3

FIG. 4
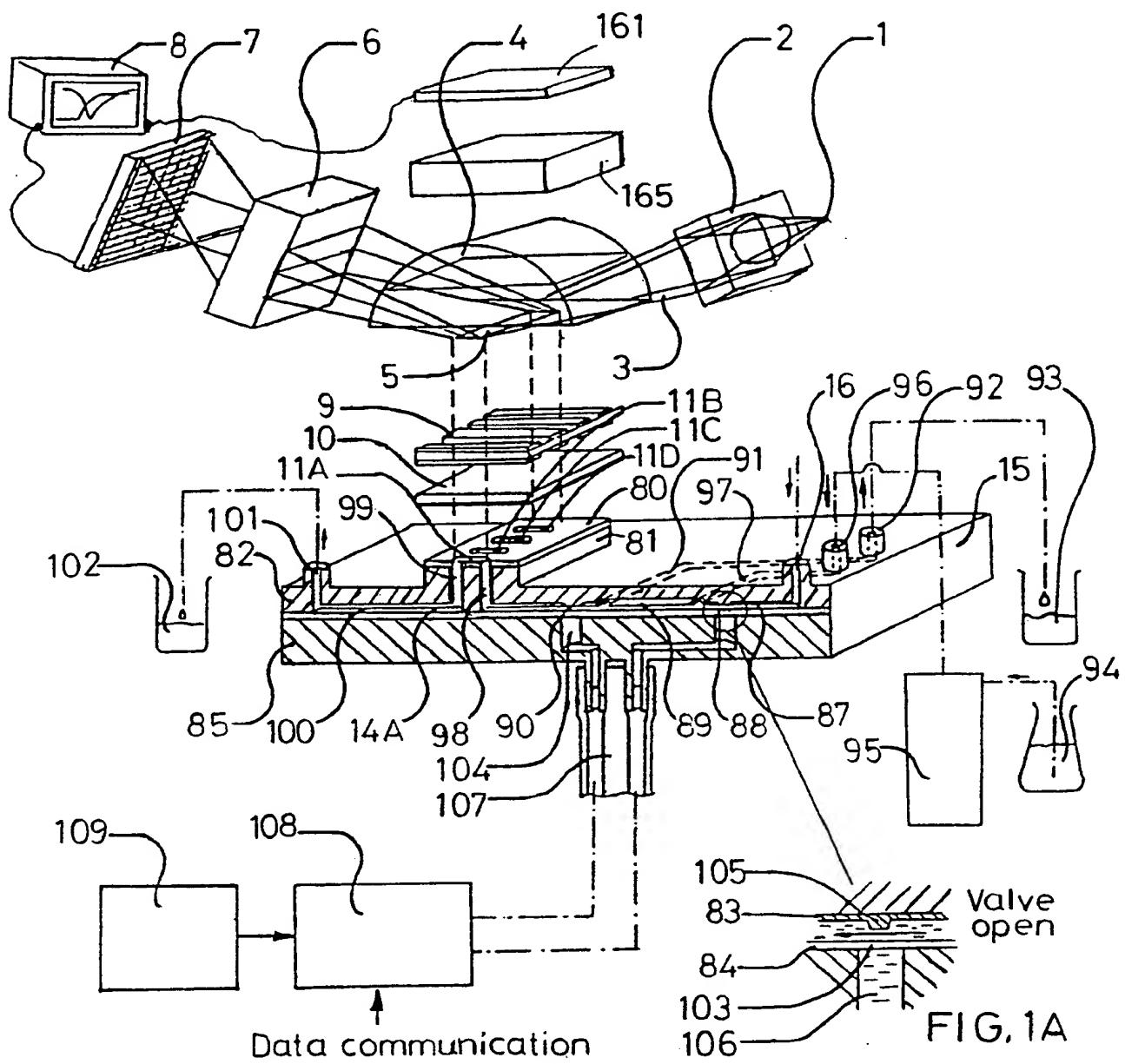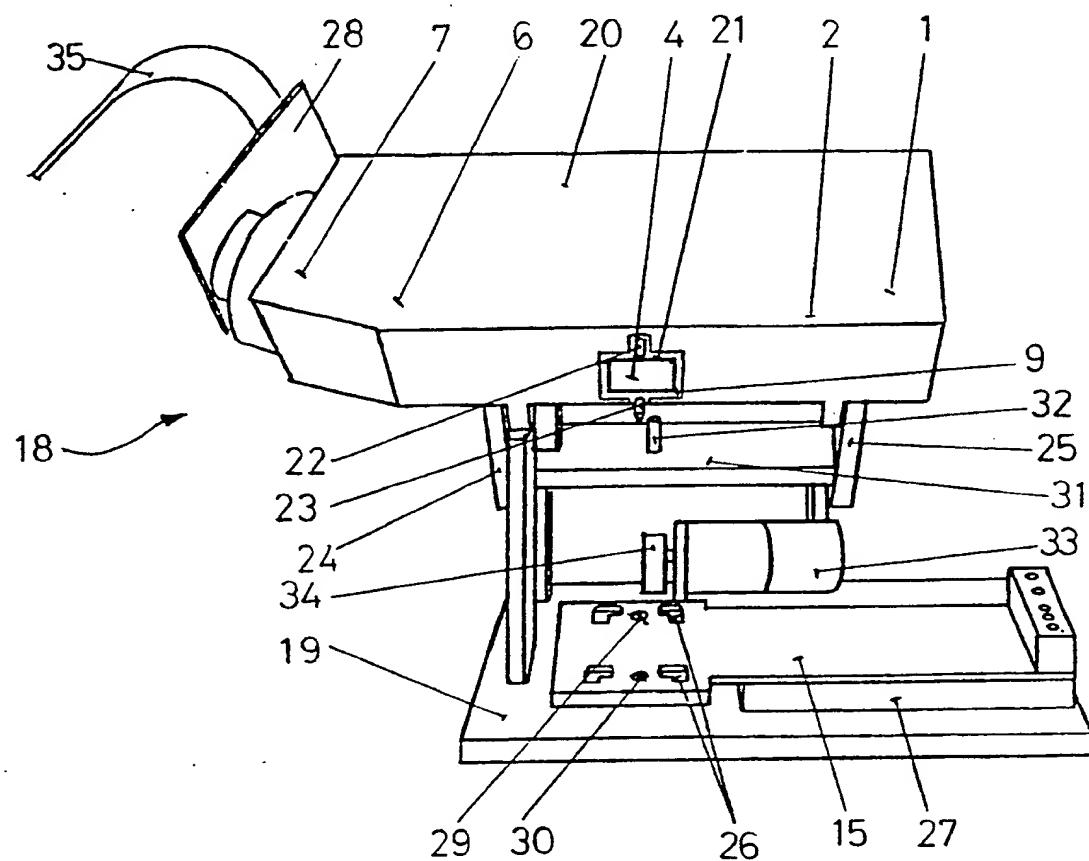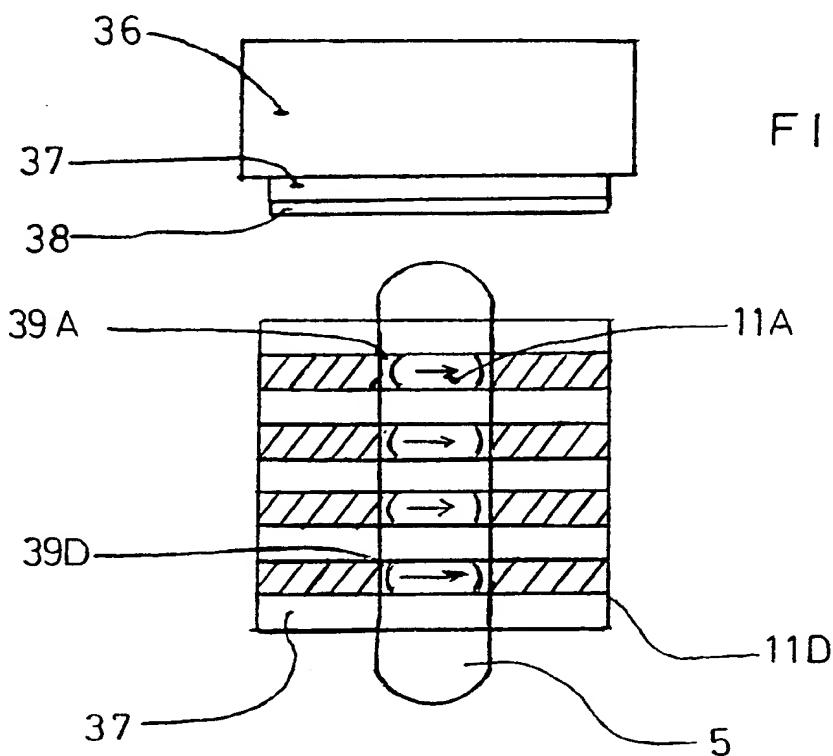
4/13
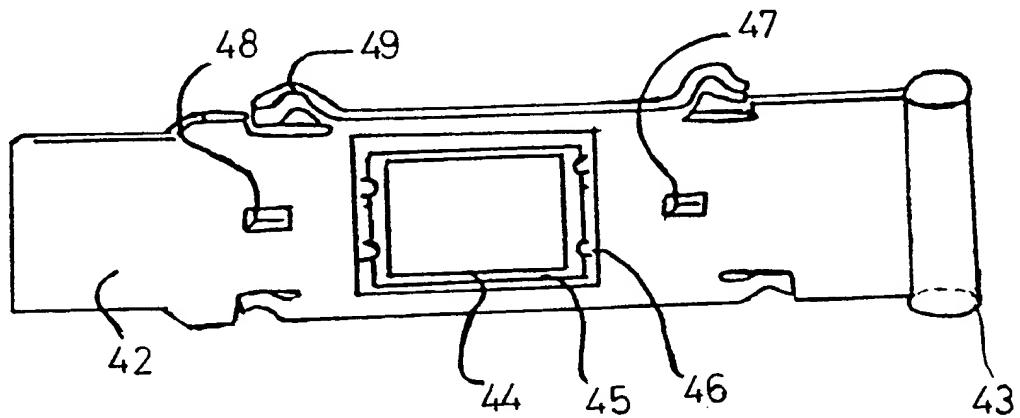
FIG. 5
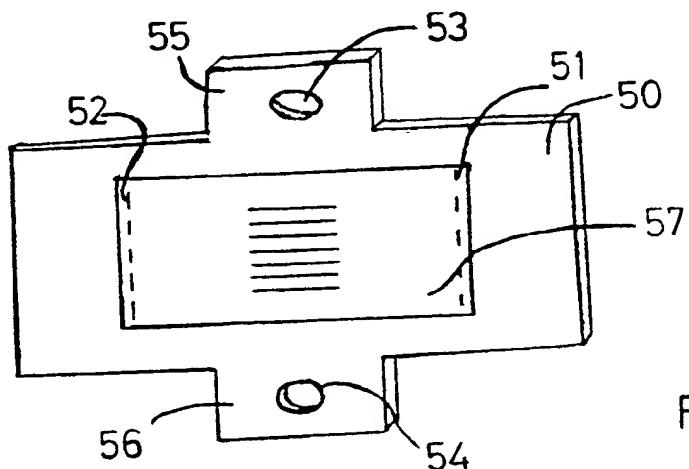
FIG. 6
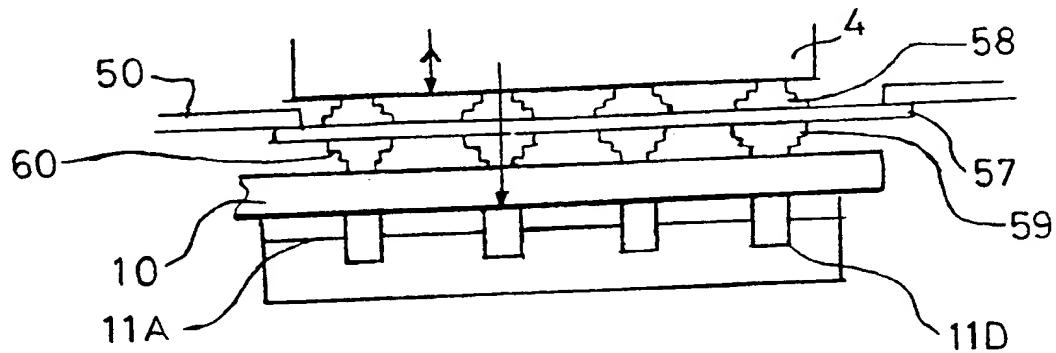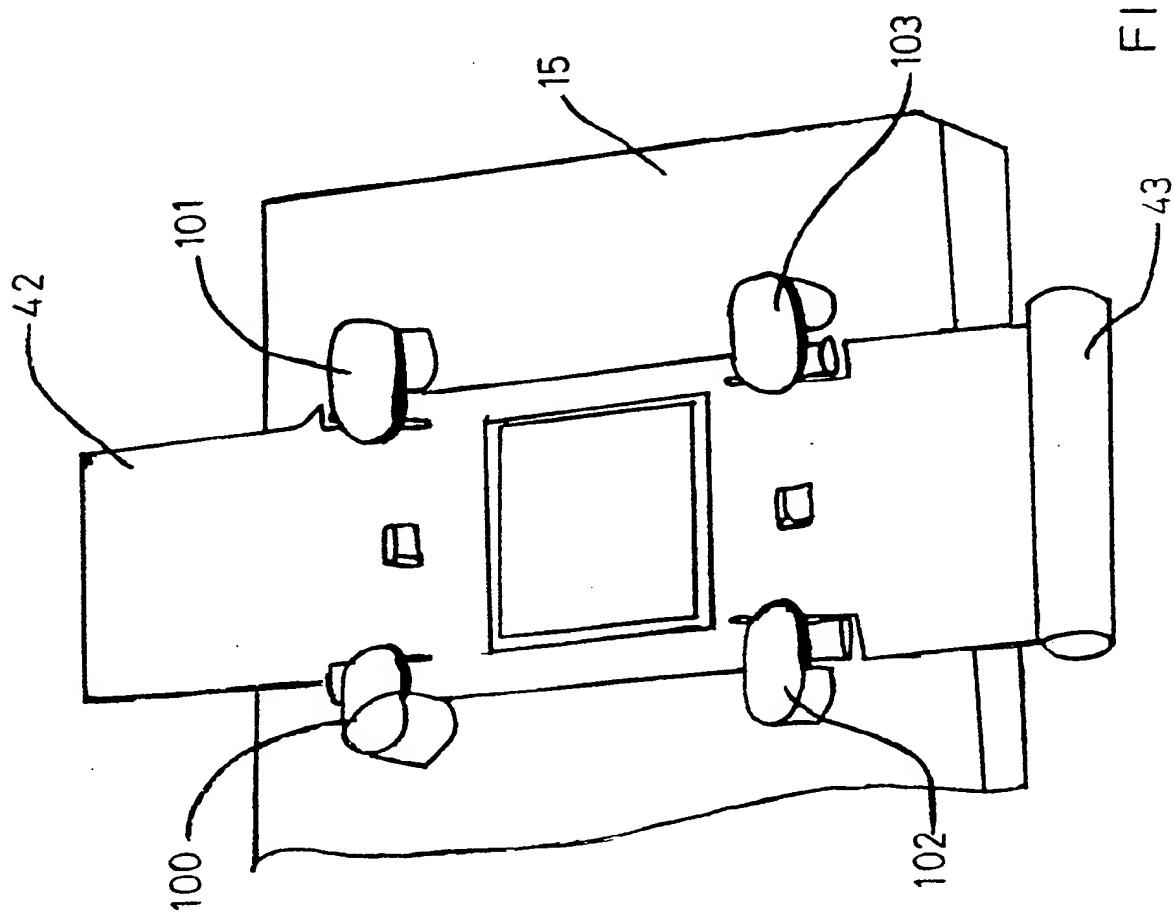
FIG. 7

SUBSTITUTE SHEET

FIG. 8
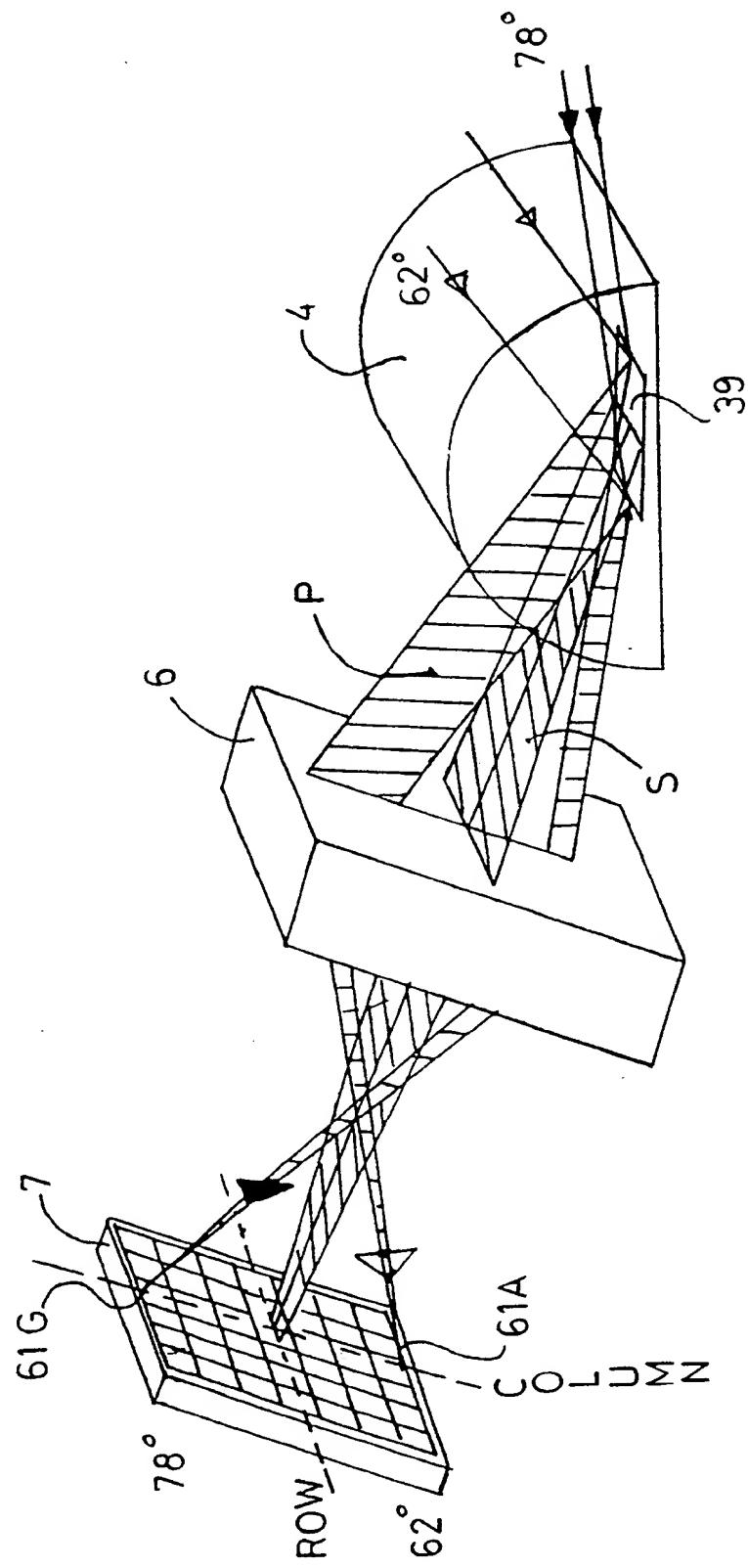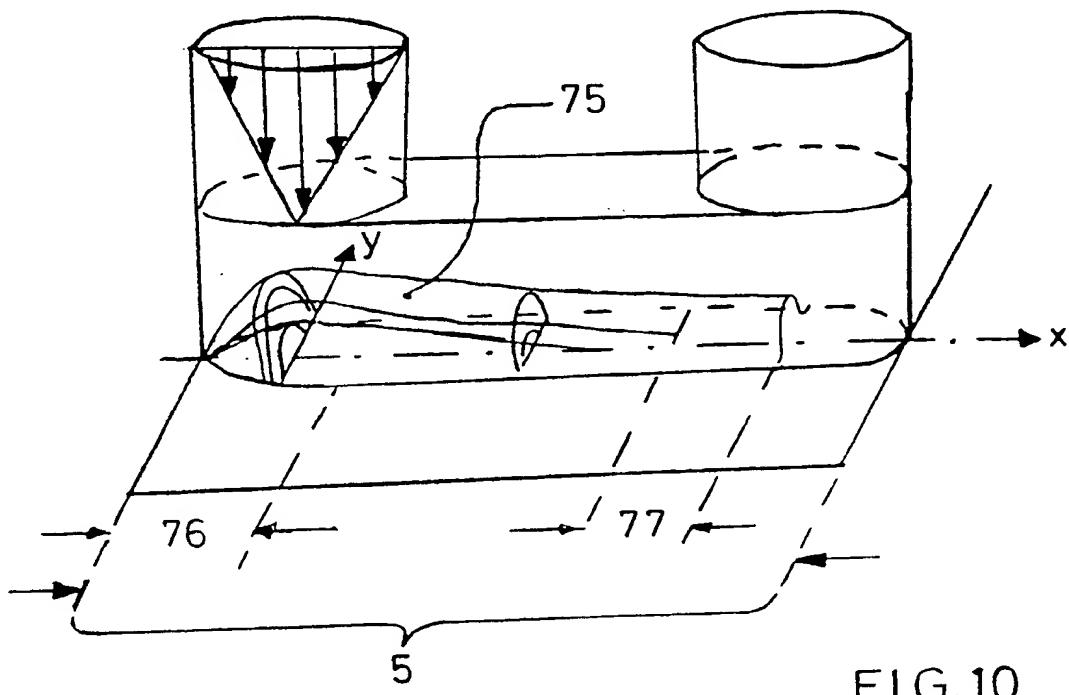
FIG.10
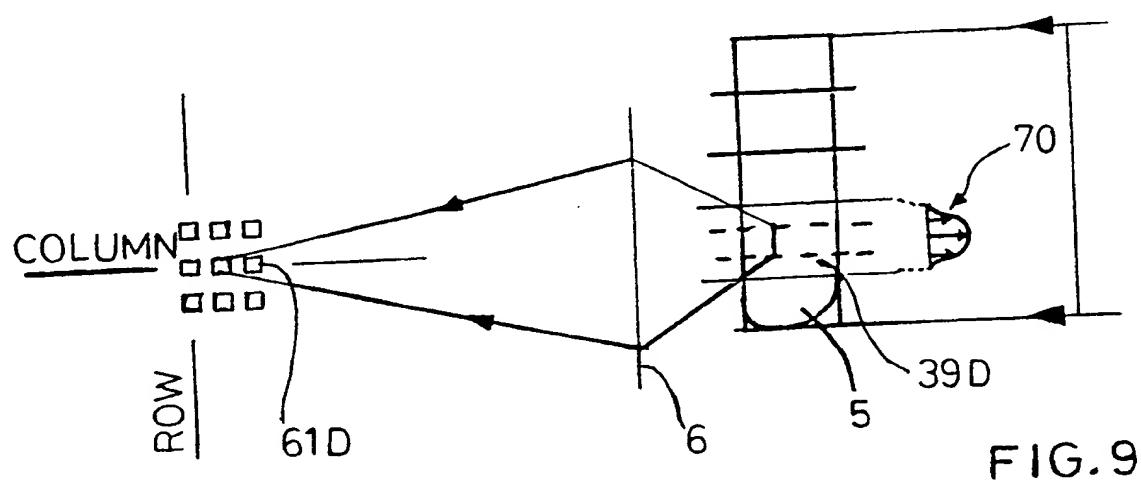
FIG.9

SUBSTITUTE SHEET
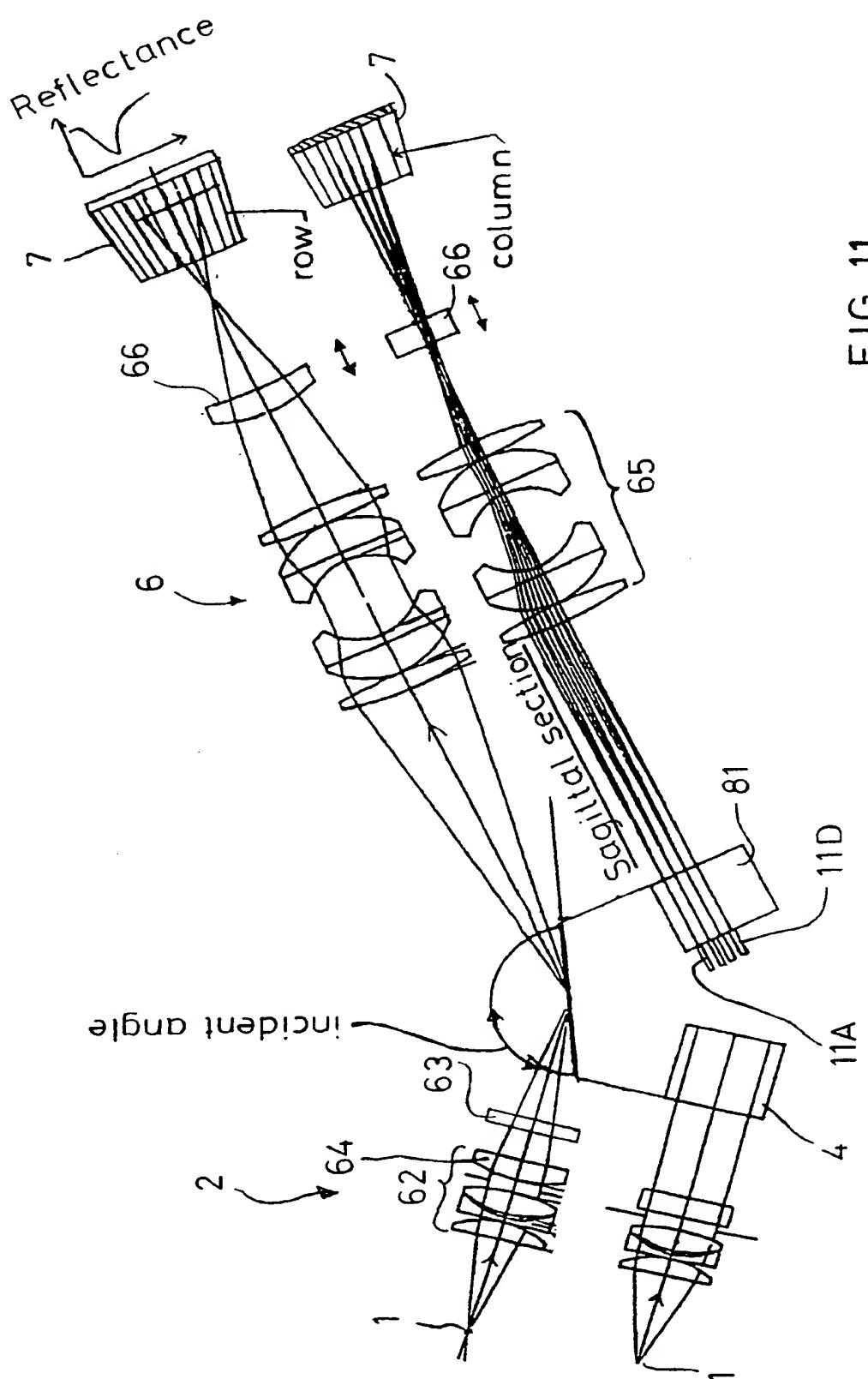
FIG. 11
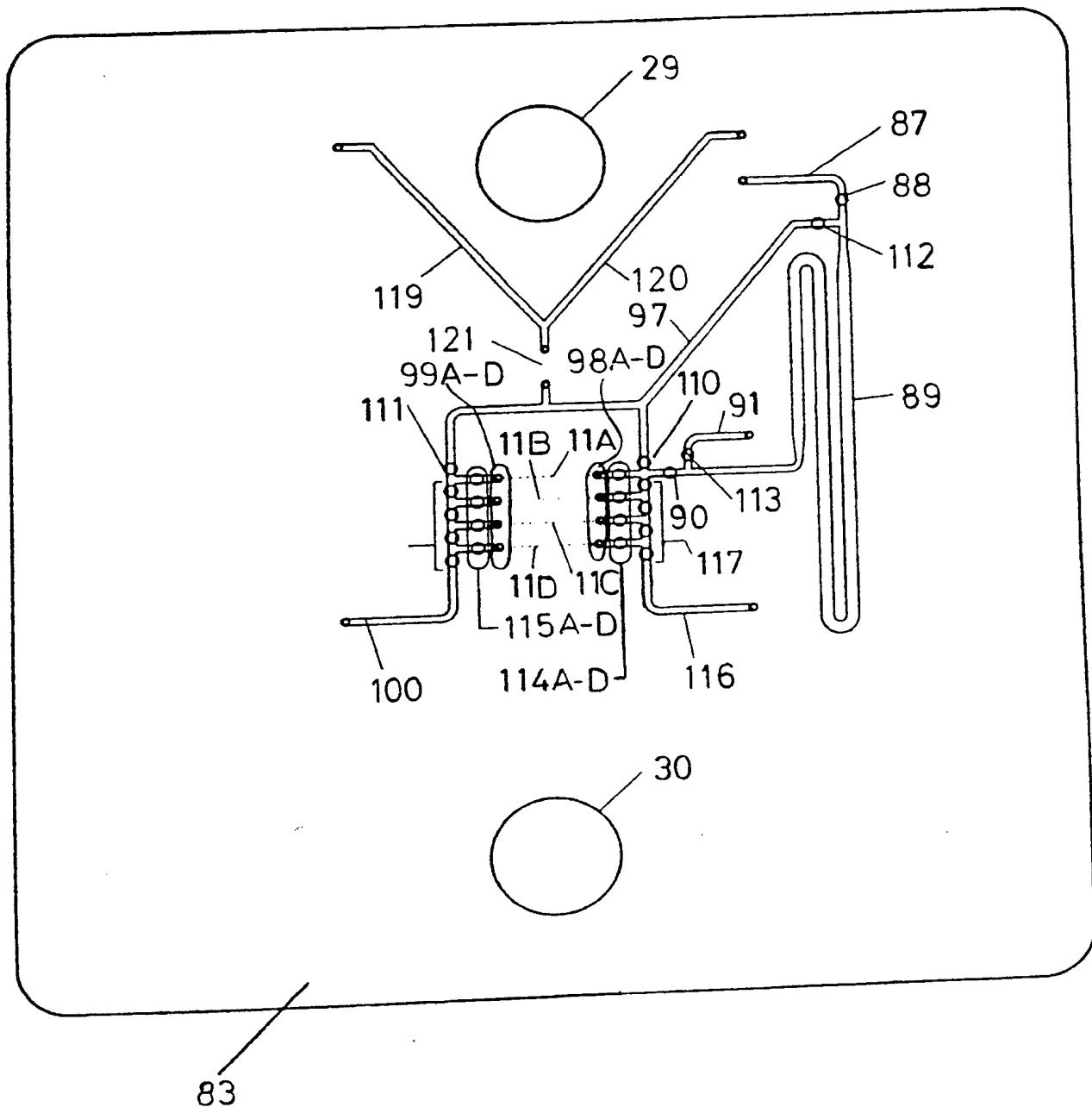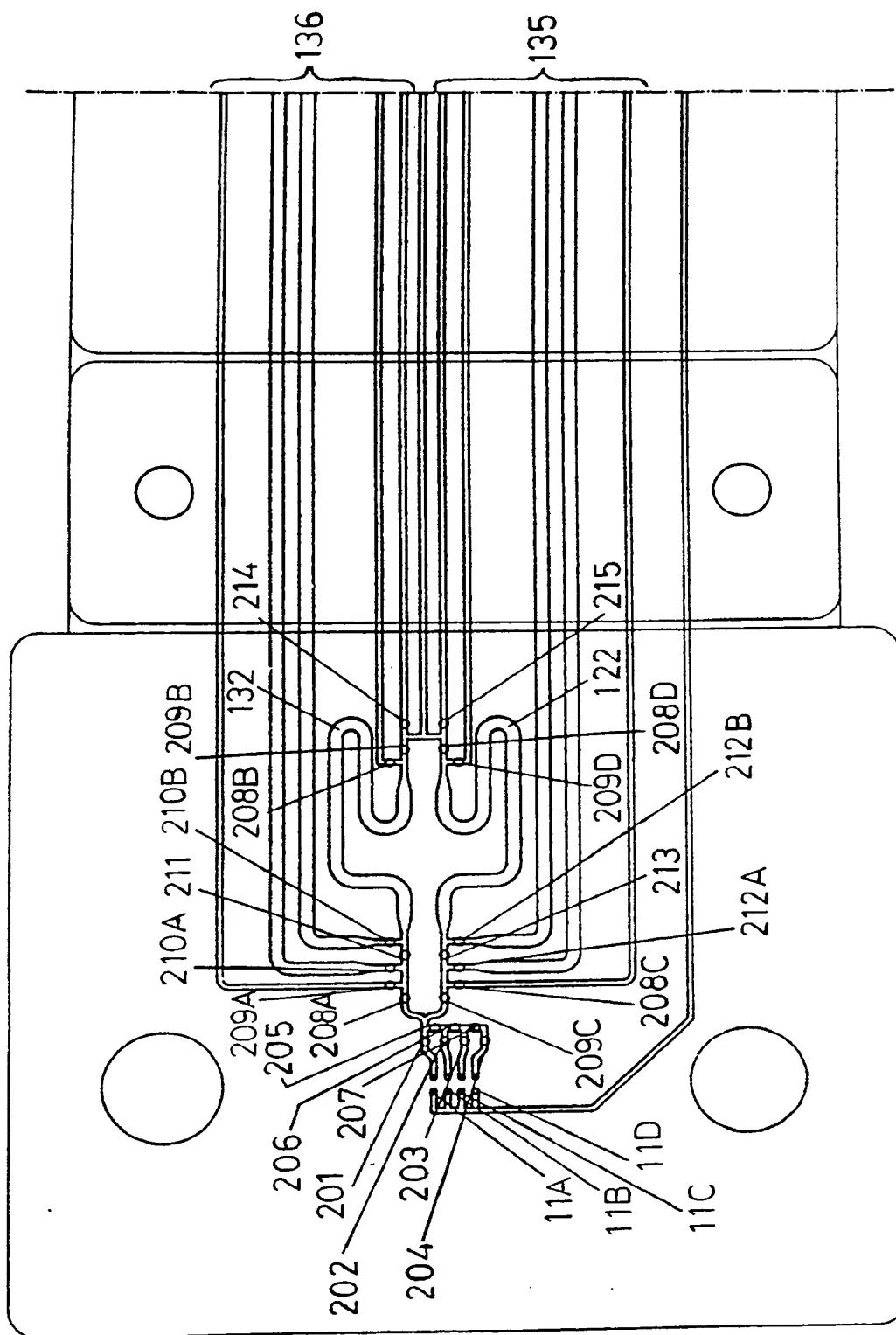
FIG. 12

FIG. 13

FIG. 14A

FIG. 14B
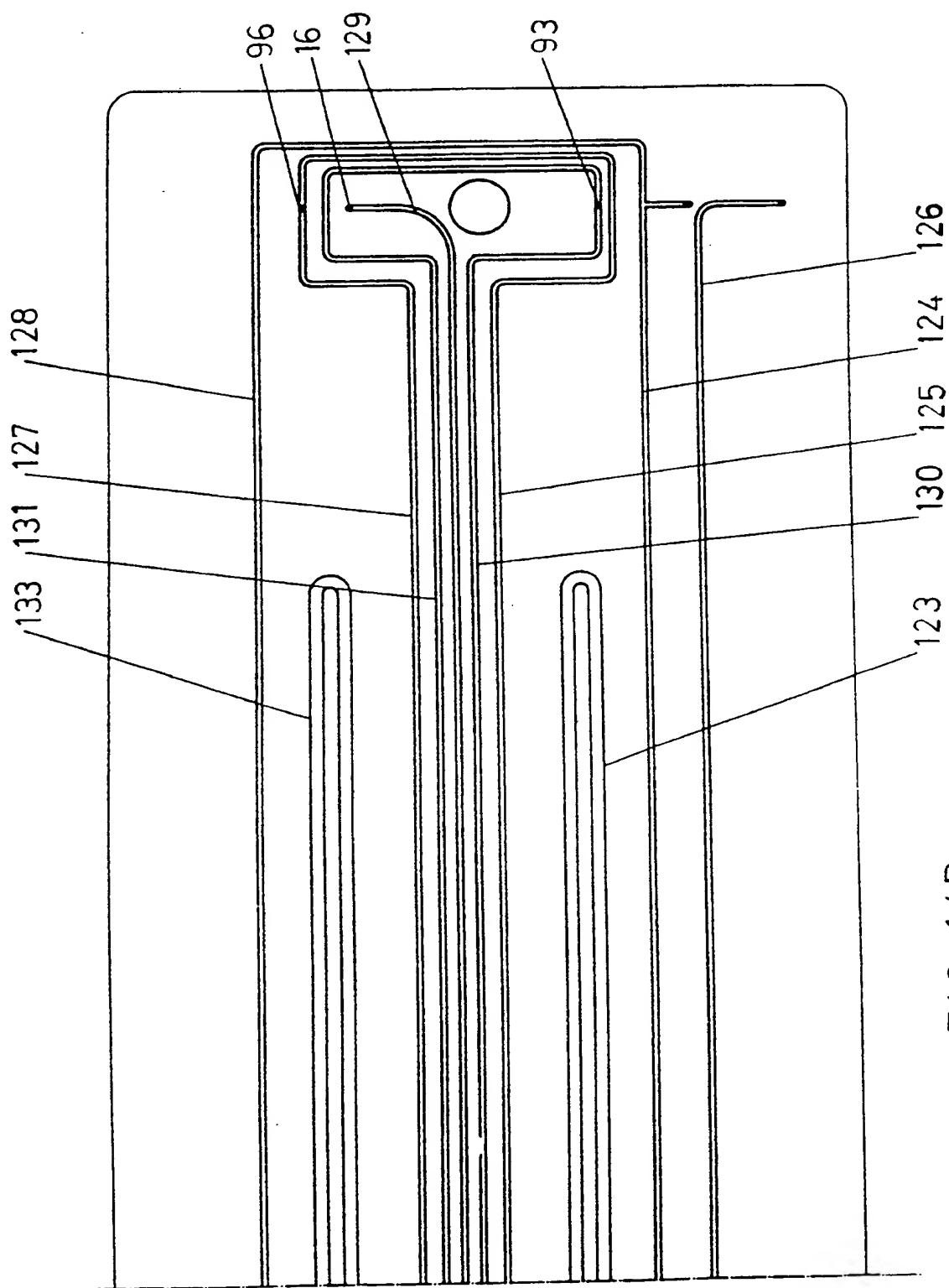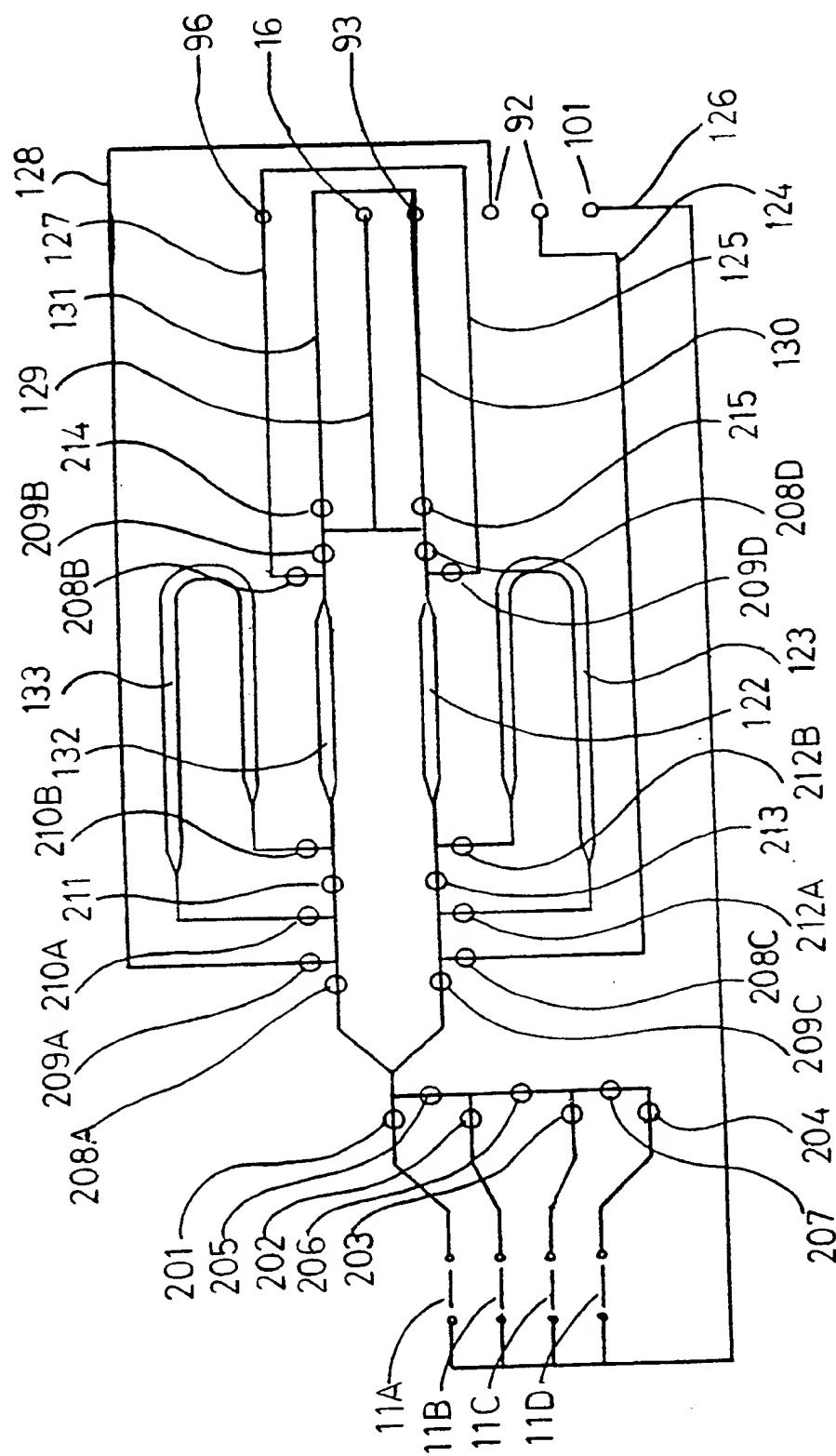
FIG. 15

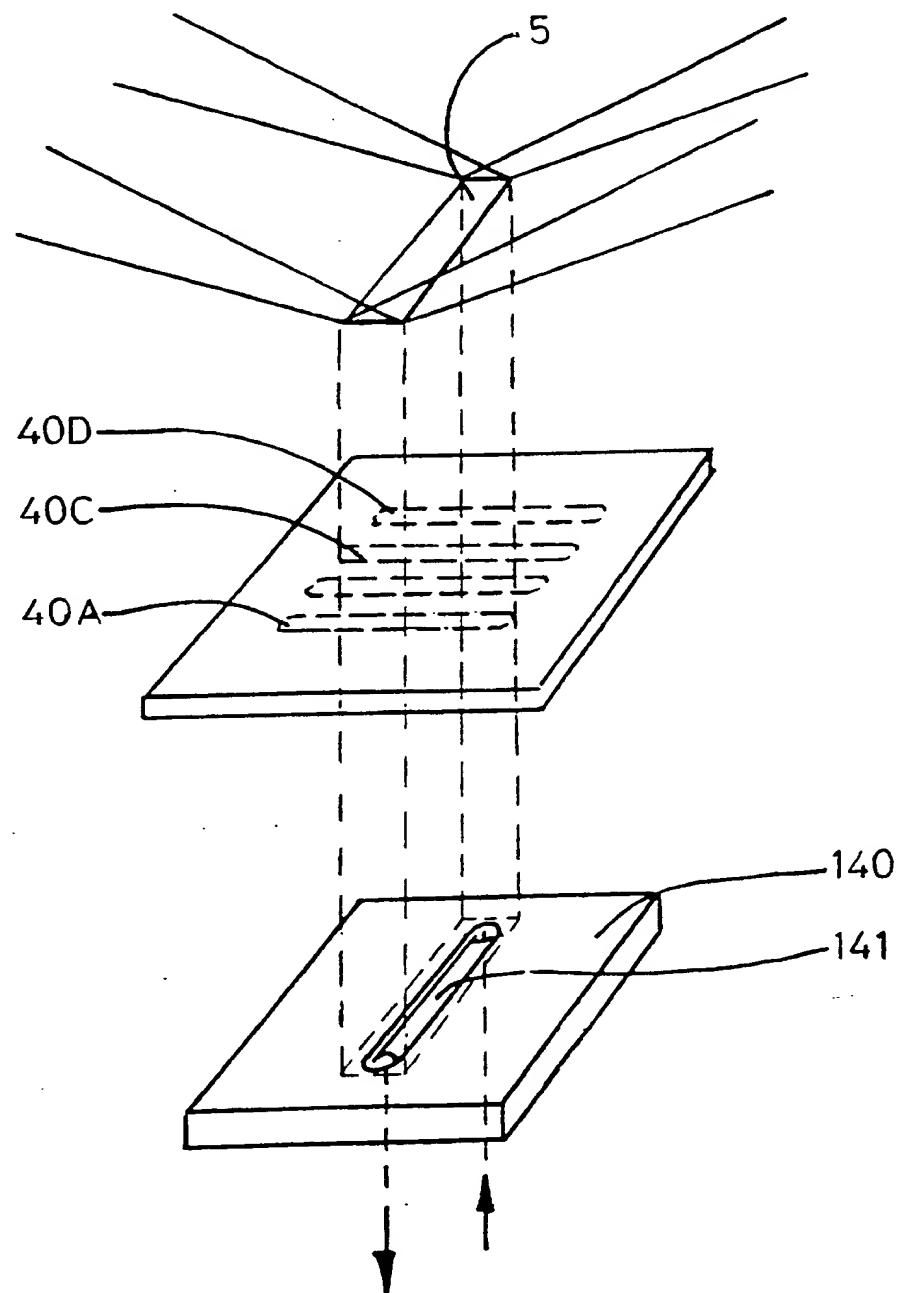FIG. 16

INTERNATIONAL SEARCH REPORT

International Application No | PCT/SE 89/00641

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 21/17, 33/53

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System		Classification Symbols
IPC5		G 01 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched *

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
A	GB, A, 2197065 (STC PLC) 11 May 1988, see the whole document	1-43
A	GB, A, 2197068 (STC PLC) 11 May 1988, see the whole document	1-43
A	EP, A3, 0257955 (AMERSHAM INTERNATIONAL PLC) 2 March 1988, see the whole document	1-43
A	EP, A2, 0202021 (PLESSEY OVERSEAS LIMITED) 20 November 1986, see the whole document	1-43

* Special categories of cited documents: **

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

21st January 1990

Date of Mailing of this International Search Report

1990 -01- 2 4

International Searching Authority

SWEDISH PATENT OFFICE

Signature of Authorized Officer

Karin Säfsten